CAPTIVE AND WILD OBSERVATIONS OF THE COURTSHIP AND SPAWNING BEHAVIOR OF GUADALUPE BASS *MICROPTERUS TRECULII*

A Thesis

by

EDWARD JAMES ENRIQUEZ

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Chair of Committee,	Frances P Gelwick
Co-Chair of Committee,	Jane M Packard
Committee Member,	Gerard T Kyle
Head of Department,	Michael P Masser

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ABSTRACT

The Guadalupe Bass *Micropterus treculii* is native to the Texas Hill Country of central Texas. It is distributed across portions of the Brazos, Colorado, Guadalupe and San Antonio River basins. The Guadalupe Bass is threatened by hybridization with non-native smallmouth bass *Micropterus dolomieu*. Because pure bred Guadalupe Bass are increasingly harder to find in areas where Smallmouth Bass have become established, Texas Parks and Wildlife Department developed a captive spawning program for Guadalupe Bass for supplemental stocking to counter the influence of hybridization. To determine potential mechanisms influencing hybridization in Guadalupe Bass populations, objectives were: (1) characterize nesting habitat and behavior, (2) compare courtship behaviors in native and hatchery environments, and (3) identify behaviors potentially influencing genetic diversity in offspring of hatchery fish. Initial field studies included snorkeling and visual surveys conducted during September and November in 2012 in Gorman Creek, South Llano River, Pedernales River, and the Guadalupe River; no active nests or reproductive adult Guadalupe Bass were observed. During February through May of 2013, visual surveys were repeated at the four field sites. In addition courtship and spawning behavior of Guadalupe Bass were recorded at A.E. Wood Hatchery. In both hatchery and natural environments, previously undocumented reproductive behaviors were observed. Breeding color patterns of males and females became more differentiated, aggressive behavior of males included opercular flaring and jaw locking, and individual males courted multiple females simultaneously and syntopically at nest sites. Only 50% of the available nesting mats were used because of

the monopolization of females and nest sites by a few males. Frequencies were significantly different for several male and female behaviors when comparing hatchery to natural environments. The prototypical courting and spawning sequence of the Guadalupe Bass were visually represented in kinematic diagrams. Although behavioral sequences were similar in hatchery and natural sites, certain transition probabilities differed. Potential mechanisms for hybridization were: similar color pattern changes in males and females of both species, female preference in both species for larger males, similar use of nesting habitat in both species, similar reproductive behavioral repertoire, and the monopolization of nesting sites and females by males.

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NOMENCLATURE

TPWD	Texas Parks & Wildlife Department
GC	Gorman Creek
PR	Pedernales River
DO	Dissolved Oxygen

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1. INTRODUCTION

The Guadalupe Bass *Micropterus treculii* is endemic to the Edwards Plateau region of Texas in the Brazos, Colorado, Guadalupe and San Antonio River drainages (Koppelman and Garrett 2002; MacCrimmon and Robbins 1975; Page and Burr 2011). The Guadalupe Bass inhabits swift moving rivers and streams within its native range of the Edward's Plateau region of central Texas (Hubbs 1957). Guadalupe Bass are adapted to small stream or small river environments and do not survive well in lentic systems (Hurst et al. 1975; Edwards 1980). It is a member of the family *Centrarchidae*, within the genus *Micropterus,* which also are known as the "Black Basses". This genus includes three more widely recognized species; Largemouth Bass *M. salmoides*, and Smallmouth Bass *M. dolomieu,* and Spotted Bass *M. punctulatus*. The Guadalupe Bass is similar in appearance to the Spotted Bass except the Guadalupe Bass has 10-12 dark bars along its side and a caudal spot (Page and Burr 2011). The Guadalupe Bass can attain a maximum size of 1.67 kg and average size is 0.5–1 kg (Koppelman and Garrett 2002). This species is growing in popularity as a sport fish for fly fisherman and anglers using ultra-light tackle (Boyer et al. 1977; Koppelman and Garrett 2002).

The Texas Parks and Wildlife Department (TPWD) began stocking Smallmouth Bass in central Texas with the goal of improving sport-fishing opportunities within the Texas hill country (Garrett 1985; Koppelman and Garrett 2002). As early as 1982 it was confirmed that hybridization and introgression were occurring in Guadalupe Bass populations where non-native Smallmouth Bass had been introduced (Whitmore and Butler 1982; Whitmore 1983). Hybridization is defined as the interbreeding of

individuals from two genetically distinct populations, and introgression is defined as gene flow between populations whose individuals hybridize and backcross with one or both parental populations (Rhymer and Simberloff 1996). Stocking of Smallmouth Bass within the Guadalupe Bass native range has ceased (Koppelman and Garrett 2002), although it continues in reservoirs throughout Texas (TWPD Stocking Report 2013). The conservation status of Guadalupe Bass is classified as vulnerable throughout its native range (Warren et al. 2000; Bonner and Bean 2008; Jelks et al. 2008). Previously reported pure strain populations of Guadalupe Bass included: Pedernales River, Gorman Creek (Colorado River Drainage) and the San Saba, Llano, and Medina Rivers (Garrett 1991; Koppelman and Garrett 2002).

Species specific coloration and behavioral cues are thought to play a role in mate choice and can form a barrier to interbreeding between two species (Wong et al. 2004; Williams and Mendelson 2011). Also, changes in color patterns of males and females during courting bouts have been observed in several species of *Micropterus* (Warren 2009). Nesting habitat selected and used by fish can also maintain barriers to interbreeding between two species. Although hybridization with Smallmouth Bass has been documented (Bean et al. 2013; Edwards 1980; Littrell et al 2007; Whitmore and Butler 1982; Whitmore 1983), no detailed study of courtship and spawning behaviors, of Guadalupe Bass or habitats used for nesting sites, have been published for Guadalupe Bass (Warren 2009).

Single stocking events of pure strain Guadalupe Bass fingerlings have not been effective at reducing the incidence of hybrids between Guadalupe Bass and Smallmouth

Bass. For example, a total of 80,000 pure strain Guadalupe Bass were supplementally stocked in 1994 and 1995 by TPWD into the Blanco River population (Littrell et al. 2007). In 2007, genetic samples of Black Basses from the Blanco River revealed that 40% of individuals were pure Smallmouth Bass, 51% were Smallmouth Bass X Guadalupe Bass hybrids, and 9% a mix of *Micropterus* hybrids (Littrell et al. 2007). Despite the introduction of pure strain Guadalupe Bass into the Blanco River they were not able to persist in a system where Smallmouth Bass and hybrids were present. The Texas Parks and Wildlife Department evaluated the potential for an intensive stocking program focused on hatchery production and supplemental stocking of pure strain Guadalupe Bass into hybridized populations. The success of a stocking effort would be the reduction of hybridization within Guadalupe Bass populations to ≤ 1% (Allendorf and Leary 1988).

The initial breeding and raising of pure strain Guadalupe Bass took place at the Heart of the Hills Fisheries Science Center in Mountain Home, Texas. This facility which is near Johnson Creek, served as the launching pad for the pilot program to determine if repeated annual stocking events would reduce the rate of hybridization within a system. Stocking of pure strain Guadalupe Bass into Johnson Creek began in 1992 and continued annually until 2007. Electrophoretic analyses of this population has showed a significant decrease in the rate of hybridization from approximately (15-30%) to approximately 1% (Koppelman and Garrett 2002; Fleming et al. 2013). Bolstered by this success, TPWD expanded introductions of pure strain Guadalupe Bass throughout the Guadalupe River, and to the western edge of its range in the South Llano River.

Subsequently, production of Guadalupe Bass for restoration was moved to the larger facility at the A.E. Wood Hatchery in San Marcos, Texas. The protocol developed by TPWD for raising Black Bass is outlined in Glenewinkel et al. (2009).

Hatchery produced Guadalupe Bass are currently stocked into the South Llano River near Junction, Texas, which is also the site for intensive habitat restoration efforts (T. Grabowski and T. Arsuffi, Texas Tech University, personal communication 2013). The brood stock are pure strain individuals that were collected from the upper South Llano River in 2010 (C. Thibideaux, Texas Parks and Wildlife Department, personal communication 2013). Captive observations and genetic data suggest that limited numbers of males are successfully mating with a majority of the females in hatchery raceways (Lutz-Carrillo et al. 2013). For example Lutz-Carrillo et al. (2013) found that 34% of males and 82% of females participated in spawning events within a raceway during the spring of 2012. Males acquired 3 to 7 mates, whereas females acquired only 1 to 2 mates (Lutz-Carrillo et al. 2013). No detailed observations of the courtship and spawning behavior were made within the captive setting. It is unclear whether this is typical behavior for Guadalupe Bass in a natural system, or due to the particular conditions of the hatchery system.

Such unequal ratios of parental contribution to offspring could reduce the effective population size of fry produced for the reintroduction initiative to genetically swamp out hybrid Smallmouth Bass X Guadalupe Bass individuals. The effective population size as opposed to the observed total population size is what determines the loss of genetic diversity and inbreeding within captive populations (Frankham et al.

2010). As effective population size decreases genetic drift reduces genetic variation and elevates the probability of deleterious alleles, and reduces the effectiveness of selection (Hare et al. 2011).

In general, to ensure that genetic diversity is not lost, it is imperative to maintain an adequate effective population size in the hatchery setting (Hare et al. 2011). Supplementation by "fresh" wild caught Guadalupe Bass could alleviate this threat by providing an infusion of new genetic material. A second method to reduce the loss of genetic diversity would be to remove dominant males to allow less dominant males to contribute to the production of fry for reintroduction efforts (Fiumera et al. 2000). Steelhead Trout *Oncorhynchus mykiss* raised in the hatchery and released into the wild showed a reduction in reproductive capability by ~ 40% per captive-reared generation (Araki et al. 2007). My observations and analyses of Guadalupe Bass courtship and spawning behavior at A.E. Wood Hatchery and a natural setting were intended to better understand the potential for differences in genetic contribution as a consequence of the current restocking program.

Among male Smallmouth Bass studied by Hanson and Cooke (2009) total length and body stoutness were the only reliable predictors of female mate choice, whereas nutritional indicators were not significant predictors. Parental-care theory suggests that if energy level of the parental male decreases during care of offspring to a point that will threaten the male's future potential for reproduction (or increase potential for death), then the individual should abandon its current brood (Trivers 1972; Sargent et al. 1986).

Thus, female choice of male partners may be biased towards larger and stouter individuals as indicators of greater brood success.

Micropterus basses are described as sexually monomorphic, that is male and female individuals are similar in appearance and have no external distinguishing characteristics (Warren 2009). Thus, several mechanisms could underlie the persistence of hybrid individuals. Natural hybridization among *Micropterus* basses is well documented (Morizot et al. 1991; Pipas and Bulow 1998; Whitmore and Hellier 1988). Temporary color changes occur in males and females of other species of *Micropterus* during the spawning season (Breder and Rosen 1966; Heidinger 1975; Miller 1975; Trautman 1981; Williams and Burgess 1999). However no detailed study of reproductive behaviors have been published for Guadalupe Bass. Color pattern changes have been documented for Smallmouth Bass; females develops dark vertical bars, and the rest of the body becomes lighter in coloration, whereas males darken and remain one color overall, as the vertical barring fades (Breder and Rosen 1966; Schneider 1971; Ridgway et al. 1989).

If color pattern is irrelevant in female mate choice, but larger male body size is relevant, then large males of Smallmouth Bass should be chosen by female Guadalupe Bass over most Guadalupe Bass males where they co-occur, because male Guadalupe Bass are typically smaller in size than male Smallmouth Bass (Warren 2009). Female Guadalupe Bass may be making the best choice for the survival of their young, and thus their own genetic fitness, but making the wrong choice in regards to the genetic integrity of the Guadalupe Bass population. These factors may contribute to the continued

persistence of hybrid forms and the decline of pure strain Guadalupe Bass across its native distribution in central Texas. Documenting courting and spawning behavioral sequences of Guadalupe Bass in the captive and natural setting contributes information to answer such questions regarding the process and consequence of mate choice to populations of this unique species. Video recordings of courting and spawning can be used to document the relative size of male Guadalupe Bass that court and spawn with female Guadalupe Bass. Thus, video should be an effective method of collecting and reviewing data for courtship, spawning, and parental care behaviors of Guadalupe Bass, in both the hatchery and in natural populations.

General descriptions of the length of the spawning season for Guadalupe Bass are between March and September (Boyer et al. 1977; Edwards 1980), although (Edwards 1980) observed that most spawning activity was from March through June. A secondary spawning event may occur later in the year after the typical spring spawning season (Edwards 1980; Edwards 1997), but has not been documented. My study provides additional information regarding secondary spawning events. Boyer et al. (1977) observed one Guadalupe bass nesting site in the Guadalupe River, which was within a pool environment in the Guadalupe River at a depth of 69 cm, 1 m from shore, and on a sloping bank. The nest was oval shaped (41× 50 cm, 10 cm in depth) where the stream bottom had been swept away leaving hard black soil with rubble, and was partially covered with sticks and leaves (Boyer et al. 1977). I compared this habitat used by Guadalupe Bass in other river systems.

Nesting density of Guadalupe Bass in the natural environment has not been described, but has been described for several other species of *Micropterus*. In riverine habitats Smallmouth Bass nest density was 3 nests per 100 m, although average internest distances of 4.2 m have been reported (Surber 1943; Pflieger 1966; Coble 1975; Lukas and Orth 1995; Knotek and Orth 1998). Spotted Bass nesting density was reported as 0.5-11 nests per 100 meters of shoreline (Warren 2009); Largemouth Bass constructed nests at an average internest distance of 6.2-9.4 m in a reservoir in Arkansas or 15 nests per 100 meter transect (Hunt and Annett 2002). Thus, inter-nest distance is an important element of site selection in the natural environment, and possibly could influence the number of successful nesting pairs in a hatchery setting. Nesting site selection may be responsible for the continued persistence of hybrid individuals in systems where Smallmouth Bass were stocked.

My study of the courting, spawning, and parental care behaviors of the Guadalupe Bass documents and describes the courtship and spawning of Guadalupe Bass in both a captive setting and in a wild population. Male and female behaviors will be documented as outlined by Székely et al. (2000) as the authors identify that in order to understand the reproductive sequence, male and female behaviors should be identified and understood. Identification of nesting habitats was included. Data of special concern included: habitat type used, substrate type, and internest distances of Guadalupe Bass in a natural setting. This information will be of great useful in the management of pure strain populations of Guadalupe Bass and for the restoration of stream habitat where Guadalupe Bass were extirpated. Degraded stream and rivers systems can be managed

with attention to providing adequate and appropriate nesting habitat. Additional information collected during this study included: length of parental care (e.g. from the time eggs are fertilized until fry are dispersed), spawning period, and other environmental data in order to augment the currently sparse information available regarding reproductive life history of Guadalupe Bass.

To better understand the reproductive life history of the Guadalupe Bass, I documented and described the courting and spawning behaviors in captive and natural settings, and parental care in a natural stream. I compared characteristics of courting and spawning sequences for captive and wild Guadalupe Bass. I tested the distribution of nesting sites used by Guadalupe Bass to their availability (null) to determine if Guadalupe Bass males maximize inter-nest distance within a captive setting, and if males choose nest sites with particular characteristics as compared to a random sample of sites. I used the data collected in the wild and the captive setting to create a prototypical courtship and spawning sequence from the initiation of courting to the release and fertilization of eggs for Guadalupe Bass.

2. MATERIALS AND METHODS

2.1 Study Sites

Four study sites were selected in regards to the natural historic distribution and the genetic integrity of the Guadalupe Bass within those systems. All four sites are in state parks and within the historic native range of the Guadalupe bass (MacCrimmon and Robbins 1975; Page and Burr 2011; Koppelman and Garrett 2002). Water conditions allowed for bankside and snorkeling observations of reproductive behavior in Guadalupe bass. Gorman creek (Colorado Bend State Park; San Saba County, TX) and the Pedernales River (Pedernales Falls State Park; Blanco County, TX) contain pure strain Guadalupe Bass populations (Garrett 1991; Koppelman & Garrett 2002), whereas the South Llano River (South Llano River State Park; Kimble County, TX) and the Guadalupe River (Guadalupe River State Park; Comal County, TX) contain introgressed populations.

Gorman Creek is a 1 km tributary of the Colorado River that has not been stocked with smallmouth bass. Although the Colorado River into which Gorman Creek flows into has been stocked with smallmouth bass. A 10 m high waterfall separates Gorman Creek from the Colorado River, inhibiting direct access of Smallmouth Bass to Gorman Creek. TPWD has not stocked Smallmouth Bass into any segment of the Pedernales River (Garrett 1991; Koppelman and Garrett 2002). A recent genetic analysis of Guadalupe Bass across its range (Bean et al. 2013) confirmed that the Pedernales River populations contains only pure strain individuals.

Smallmouth Bass were extensively stocked into the Guadalupe River, and into Canyon Lake, which was impounded on the Guadalupe River downstream of my study site. The Guadalupe River has been supplementally stocked with pure strain Guadalupe Bass in an attempt to genetically swamp out hybrids within the system. Smallmouth bass were stocked into reservoirs downstream of the confluence of the Llano River with the Colorado River. Lyndon B. Johnson Reservoir (just downstream of the confluence) in 1974, 1984, 1985, and 1986 (TPWD Stocking Report 2013). The South Llano River received supplemental stockings of Guadalupe bass in 2011 and 2012.

2.2 Field Data Collection: Fall 2012

I conducted field work from June 6th through November 30th 2012. Transects along the stream bank were walked from downstream to upstream between access points within each state park boundary. I looked for active nests while walking the banks, with the aid of polarized sunglasses to reduce surface glare. I used angling as the primary collection method to target individual adult fish (Sutherland 2006) in order to determine if adult Guadalupe Bass were producing free-flowing gametes. I measured total length and weight of each fish caught, the abdomen was massaged and palpated to check for free flowing gametes (Cabrita et al. 2008), and I took digital photographs of each individual. A t-test (MS Excel) was used to compare length and weight data for a pure strain Guadalupe Bass population that had been previously fished (Pedernales River, PR) to that for a pure strain unfished population, GC).

Water quality was measured using a YSI Pro Plus multiparameter meter (Quatro Cable Model # 605790-X) at sites where Guadalupe Bass were sampled, and data were

associated with site locations using GPS (Garmin etrex 2.11). Water quality parameters included: dissolved oxygen concentration (DO, mg/L), and saturation DO saturation (%), temperature (°C), salinity (ppt), pH, and total dissolved solids (TDS mg/L).

2.3 Field Data Collection: Spring 2013

I conducted spring field work from February 4th to May 10th 2013. Sampling protocol followed that of the fall field season. Water quality was measured in conjunction with observed early life stages of Guadalupe Bass because it plays an important role in growth during the early life history of Centrarchids (DeVries et al. 2009). Low DO concentrations negatively affect behavior, growth and survival of centrarchid species (DeVries et al. 2009). Water quality was measured within the nest boundary of active nesting sites across the entire duration the early life history of Guadalupe Bass.

During the spring field season field work was focused on Gorman Creek at Colorado Bend State Park due to logistical constraints to coordinate field work with data collection at A.E. Wood Hatchery. Water clarity allowed me to observe fish from above and under water during their courting and spawning behavior, and their use of in-stream habitat. A nest was considered active if a male Guadalupe Bass maintained its position over the nest, aggressively guarding and defending the nest area from intrusion by other fish, or if a male and female pair were observed courting within or near the nest, or if eggs or fry were present in the nest. After recording the location of the nest (GPS, Garmin etrex 2.11), snorkeling commenced when I observed a pair of Guadalupe Bass engaged in courting behaviors. To record courting and spawning behaviors I used an

Olympus TG-820 digital camera to make underwater observations. Initially I used snorkeling to observe and record Guadalupe Bass spawning. However, my presence interrupted courtship and spawning behaviors by male and female Guadalupe Bass. On two separate occasions the female Guadalupe Bass disengaged in courting behavior and fled from the area. Video recording was continued by using high definition digital cameras (JVC Everio model # GZ-HM40BU) mounted on tripods on the stream bank. Focal video duration was approximately 30 minutes in length. Male-female interaction had the highest priority for video recording, followed by male-male interactions. Group-scan videos were third in priority; single individuals (male or female) had the lowest priority. At the start of each observation period, I first scanned for male-female interaction. In the event that focal individuals moved out of camera range during the video and could not be reacquired with 30 sec, the video was not used for analyses

Water quality data, nest diameter, distance from nesting site to nearest structure and distance to nearest neighboring nesting site was measured after the nesting site was vacated (i.e. fry had matured and dispersed away from the nest). Current velocity (m/s) was measured at the upstream edge of the nest (Marsh-McBirney Flow-mate 2000). Nest substrate composition was measured using a modified Wentworth classification scale (Bain 1999). Distance from each nest to the nearest permanent structure (i.e. log, boulder, tree stump) was measured from the center of the nest to the nearest edge of the structure. Distance between nests was measured from the center of each nest to its nearest neighbor using a tape measure.

The Olympus TG-820 camera was used to record the early life history stages of Guadalupe Bass in Gorman Creek. The camera was mounted on the end of a 1.5 meter long wooden dowel using a flexible mounting bracket for the camera mounted on the end. Video recordings were used to collect qualitative visual data in regards to coloration and life stage on a daily basis throughout the study period.

2.4 Data Collection: A.E. Wood Hatchery

Observations of Guadalupe Bass courtship and spawning behavior were conducted at the A.E. Wood State Fish Hatchery in San Marcos, Texas between March 8th and March 15th 2013. This hatchery produces Guadalupe Bass for TPWD's Guadalupe Bass restoration initiative intended to reduce the number of Smallmouth × Guadalupe Bass hybrids through numerical and genetic swamping (Allendorf and Leary 1988; Koppelman and Garrett 2002). Hatchery staff informed me that Guadalupe Bass in the raceways are extremely sensitive to human presence. Therefore, observations were made from a mezzanine platform approximately 10 to 12 m above and 3 to 4 m away from the raceway wall (Figure 1). Because of this viewpoint, the nesting mats closest to me were hidden by the raceway wall and therefore not observable. Data recorded for observed nesting mats were dates of collection of fertilized eggs by hatchery technicians. The raceway hatchery set-up (Figure 1) is not to scale, but is provided to contextualize the hatchery environment and the vantage point from which I made observations.

Raceway dimensions were 26.2 m long, 2.4 m wide, and 1 m deep. A water permeable divider was placed 11 meters from one end of the raceway to divide raceway No. 2 (used for video data collection) into two sections hereafter referred to as sections

2-1 and 2-2. Section 2-1 was 15.2 m long and section 2-2 11 m long. A total of 20 nesting mats (Spawn-Tex 45 X 60 cm) were placed into raceway No. 2. Section 2-1 received 12 mats, and section 2-2 received eight mats. The arrangement of the dividing slots built into the concrete raceway did not allow for an equal distribution of nesting mats.

In total, 42 Guadalupe Bass were placed into the raceway; 22 were placed into raceway section 2-1 and the remaining 20 were placed into raceway section 2-2. The resulting male: female ratios were 1:1.2 1:1 in sections 2-1 and 2-2. Fish were placed into the raceway on March 8th and allowed to acclimate for three days, and then spawning mats were provided on March 11th; as nesting substrate and each nesting mat site was uniquely numbered (2-1, 2-2, 2-3….2-20). The A.E. Wood Hatchery facility uses water directly pumped from the San Marcos River. Water quality was recorded during the morning and afternoon by TPWD technicians.

Two video cameras (JVC Everio model # GZ-HM40BU), each mounted separately on a tri-pod, were directed at each half of raceway 2 (Figure 11). Focal videos and scans of the raceway were the primary means of collecting behavioral data. I opportunistically documented observations of any unique behaviors that occurred in other raceways other than raceway No. 2. Focal videos (3-min duration) were concentrated on individual mats in the raceway. Video recording hierarchy for interactions between male and female Guadalupe Bass was followed in A.E. Wood Hatchery as outlined the previous section. In the event that TPWD staff disturbed a

courting pair of Guadalupe Bass, the focal was stopped and fish were allowed time to resume courting before video recording was restarted.

In addition to focal nest videos I scanned raceways by starting at the furthest nesting mat to the left of each raceway section (2-2 and 2-14), and then moving to the next nest mat to the right until the last nest mat in a section had been viewed (until presence or absence of individuals could be tallied). Scan sampling allowed me to determine which nesting mats were being used consistently throughout the study period by male Guadalupe Bass. Observation periods were conducted during the morning and afternoon based on information from TPWD hatchery staff that these were the times of day when they had observed the most sexual activity. Morning observations were made from approximately 7:00 AM, to 12:00 PM. Afternoon observations were made from approximately 2:00 PM, to when timers shut off the hatchery lighting (~6:30-7:00 PM). TWPD hatchery staff recorded temperature and DO twice a day (during the morning and again in the afternoon). These data were provided for my study by Chris Thibideaux for use in my study. In addition to water quality data provided for my study; digital photographs of Guadalupe Bass eggs, and fry were provided to compare the photographs and video recordings of Guadalupe Bass in the wild (Gorman Creek).

2.5 Ethogram

An ethogram is a natural starting point for any type of ethological research, particularly species oriented research such as this study (Lehner 1996), because it is a comprehensive catalogue of detailed descriptions of the characteristic behavior patterns of a species (Martin and Bateson 2009). The ethogram for Guadalupe Bass will focus on

reproductive behaviors. However, other behaviors will be recorded and interpreted in addition to those primary behaviors at the focus of this study. Because there was no previous information regarding courtship and spawning of Guadalupe Bass, an ethogram for Smallmouth Bass, and descriptions of male guarding behavior in smallmouth bass (Ridgway 1987; Ridgway et al. 1989; Winemiller and Taylor 1982) were used as a template for describing courtship, spawning and parental care behaviors in Guadalupe Bass.

Guadalupe Bass behaviors were divided into three behavioral activity states: conflict, sexual, and parental care. Conflict behaviors were defined as aggressive interactions amongst male Guadalupe Bass; sexual activity states were defined as interactions between male and female Guadalupe Bass; parental activity states were behaviors interpreted as actions by male Guadalupe Bass that could enhance offspring survival once eggs were deposited (Sargent et al. 1987). Each of these activity states were divided into subcategories of behaviors. Within the conflict activity state, three behavior categories are described: assess, deescalate, and escalate. The sexual activity state behavioral categories were: reject, proceptive, and receptive. Parental care activity state behavioral categories were: fanning, fanning with rotation, and escalation/attack. Within each behavior category, indicator behaviors were listed. Indicator behaviors were specific actions that the individual male or female Guadalupe Bass displayed during observations periods. Indicator behaviors from Ridgway et al. (1989) and Winemiller & Taylor (1982) were used to complete the Guadalupe Bass ethogram. Behaviors not observed in these two previous studies of Smallmouth Bass, but observed in my study of

Guadalupe Bass were included to complete the ethogram. The completed ethogram provided a repertoire of reproductive and parental care behaviors observed in the Guadalupe Bass, and was used to score behaviors in captive and wild video recordings of Guadalupe Bass courtship and spawning in order to analyze the frequency and sequences of these behaviors.

2.6 Video Data Processing

Focal video recordings (A.E. Wood Hatchery and Gorman Creek) were scored using the newly constructed ethogram. A spreadsheet was used to create a transition matrix, which then could be used to determine the frequency of observed behaviors and to calculate the behavioral transition probabilities. A transition matrix was created for each nesting mat observed at A.E. Wood Hatchery, and a transition matrix was created for each nesting site observed at Gorman Creek. Two separate types of data were extracted from these transition matrices, the frequency of each behavior observed at each nesting site in each environment (A.E. Wood Hatchery and Gorman Creek) and the transition probability data used to determine sequence order for behaviors. A separate transition matrix was scored for nesting mats 2-2, 2-10, 2-14, and 2-18 because these four nesting mat sites represented the greatest proportion (80%) of observations of courtship and spawning activity in the captive setting. In addition to comparisons among nesting mats in captivity, and to behaviors for nesting site GC-1, a cumulative transition matrix was created by combining the observed frequencies of all four nesting mats at A.E. Wood Hatchery and the nest site at Gorman Creek. Nesting site GC-1 was the only wild nesting site video recorded at Gorman Creek. Attempts to video record two other

nesting sites (GC-3 and GC-5) were not successful (courting behaviors disengaged by Guadalupe Bass).

A Chi-square goodness of fit test was used to compare behaviors among nesting mat pairs in A.E. Wood Hatchery, and to compare the matrix for each nesting mat to the cumulative transition matrix for A.E. Wood Hatchery was an accurate representation of all observed courting individuals in the hatchery. These results were used to augment analyses of the behavioral similarities and the differences between the nesting mats (A.E. Wood Hatchery), with those for wild nesting sites (Gorman Creek).

Eleven male behaviors and ten female behaviors (Table 1) are included in the transition matrices. Behaviors were different for each of the sexes with the exception of: swim away, crossover, and circling. The columns of the matrix have male and female behaviors separated by sex. The rows are behaviors that preceded the behavior in that column within a video sequence. When scoring the videos the first observed behavior is the first preceding behavior; the next observed behavior is the following behavior in the sequence. For example if contact nip was the first behavior observed in a video sequence followed by head down. The cell where the row labeled contact nip and the column labeled head down intersect is scored plus one. If this happens again during the sequence then score in the cell for this transition is increased by one. This method of scoring the sequence matrices was used on all nesting sites and pairs of Guadalupe Bass (captive and wild). Male and female behaviors are scored sequentially as they occur in "real time" within the video sequence.

During observations at both A.E. Wood and Gorman Creek I observed multiple individuals in one nest attempting to court simultaneously. For these sequences I scored only interactions between the male and the closest female within the nesting site. If two females were interacting with the male and the male was interacting with both females I recorded all behaviors as they occurred in real time during the focal video. When male and female Guadalupe bass were participating in the same behavior, but then transitioned to a new single behavior circling I scored circling as the preceding behavior for both male and female rows. For example if both the male and female are in a circling behavior, which is followed by the female body shuddering, I then scored a plus one in the cell for the male or female behavior for circling as the preceding behavior and the female column for body shuddering. All focal videos were scored using this protocol.

2.7 Data Analysis and Statistics

2.7.1 Nesting Site Use in A.E. Wood Hatchery

Largemouth Bass in a hatchery setting will occupy all available nesting mats provided to them in the raceway (C. Thibideaux and R. Sparrow, Texas Parks and Wildlife Department, personal communication 2013). For scan video recordings of the raceway were scored and the number of Guadalupe Bass (males and females) at each nesting mat were recorded. The total number of observed Guadalupe Bass was summed for the study period for each nesting mat. This data was used to determine whether Guadalupe Bass males were using individual nesting mats disproportionately to their availability by using a Chi-square goodness of fit test. Because of their separation by the mesh divider, nesting mats within raceway section 2-1 were analyzed separately from

nesting mats in raceway section 2-2. For each video scan session the observed number of male and female Guadalupe Bass per nesting mat was recorded. These observed data were used to calculate expected values for number of occupants per mats based on a null hypothesis of equal probability for distribution of fish among mats. Thus, expected number of Guadalupe Bass for each nesting mat was calculated as the total observed number of Guadalupe Bass for the section under observation, divided by the number of nesting mats available within that section.

2.7.2 Markov Chain Sequence Analysis

The sequence data from the transition matrix developed from the video recordings, were analyzed using Markov chain analysis. It is a method for distinguishing whether a behavior sequence is occurring at random or whether it contains some degree of temporal order (Martin and Bateson 2009). A zero order Markov chain model assumes that each behavior observed is independent of the previously observed behavior. That is, all behaviors have an equal chance of occurring at any point in time during the sequence (Bakeman and Gottman 1986; Lehner 1996). A first order Markov chain model assumes that the probability of occurrence of a particular behavior depends on only the immediately preceding behavior (Fagan and Young 1978; Bakeman and Gottman 1986; Lehner 1996). I tested the fit for each of these two models against the frequency of observed behaviors from Gorman Creek and A.E. Wood hatchery using a Chi-square Goodness of fit test. This information will be used to design and interpret the kinematic diagrams.

2.7.3 Behavioral Similarities and Differences of Observed Frequency Data

To test for differences in the frequency of observed indicator behaviors, and activity, log likelihood ratios and binomial z-scores were calculated from the data in the transition matrices. The results for these indices were used to compare activity states, and indicator behaviors between pairs of nesting mats in A.E. Wood Hatchery, and between each nesting mat and the nesting site GC-1 in Gorman Creek. Nesting site GC-1 was the only nesting site in Gorman Creek for which a full sequence of video observations were made successfully. Male behaviors were compared to male behaviors, and likewise female behaviors were compared to female behaviors amongst the nesting sites of captive and wild environments.

The expected frequency of a targeted behavior is calculated as the ratio of the summed total occurrences of all observed behaviors of one environment multiplied by the total occurrences of each behavior summed across the two environments being compared, and then dividing by the row totals for the targeted behavior observed summed across the two nesting sites being compared. The log likelihood ratio is calculated as the observed frequency of the targeted behavior multiplied by the natural log of the ratio of the observed frequency of the target behavior divided by the expected frequency of the targeted behavior.

The binomial z-score was calculated as the observed frequency of the targeted behavior minus the row total for that behavior multiplied by the ratio of the wild total frequency of behaviors divided by the matrix total divided by the square root of the row total multiplied by the ratio of the wild total frequency divided by the matrix total

multiplied by one minus the ratio of the total frequency of behaviors divided by the matrix total. A binomial z-score greater than ± 1.96 is considered significantly different at the 0.05 alpha level. The behaviors that produced z-scores greater than ±1.96 were significantly different from their expected frequencies and from each other across the two environments being compared.

The broader activity states: conflict, sexual, and parental care were also examined using a log likelihood test, and z-scores to determine if the frequencies of these behavioral states differed between captive and wild Guadalupe Bass. The data used in this analysis are the summed totals of observed indicator behaviors from both environments that correspond to the broader behavior activity states (Table 1). The cumulative frequency totals for each transition matrix (captive and wild nesting sites) was used for this analysis as well. Activity states at each nesting mat in A.E. Wood Hatchery were compared to the nesting site at Gorman Creek to determine if there were differences among nesting sites in the hatchery were greater than the differences between hatchery and wild nest sites. Pie charts were used to visualize the proportional distribution of the observed behaviors for male and female Guadalupe Bass from each environment (Gorman Creek and A.E. Wood Hatchery).

2.7.4 Kinematic Diagram Construction

The behavioral transition probabilities were used to create kinematic graphs, which diagram the sequence of behaviors (Blumstein and Daniel 2007), and cab be used to represent temporal or sequential order of behavioral states (Sustare 1978). I constructed two kinematic diagrams to demonstrate the prototypical courtship and

spawning sequence for Guadalupe Bass in each environment. Microsoft PowerPoint was used to create the diagram. In addition to the prototypical spawning sequence of Guadalupe Bass, I diagrammed the sequence of male-male behavior, and female-female behavior in each environment (A.E. Wood Hatchery and Gorman Creek).

The prototypical courtship and spawning sequences for captive and Gorman Creek Guadalupe Bass were diagrammed to provide a best estimate of the progression of the interactions between a male and female Guadalupe Bass where the end result is egg deposition by the female and fertilization by the male. In order to provide a visually simplified diagram, some strong behavioral transitions were omitted. For example parental care behaviors were excluded from the prototypical courtship and spawning sequence. In addition sculling and sculling with rotation behaviors had strong transition probabilities with other behaviors, they often led to "dead end" nodes for sequences in that they did not proceed to the end result of major interest for this aspect of my study. When I attempted to include all behaviors and transitions with probabilities greater than 0.10, the kinematic diagrams became too noisy to show the structure of a step by step courting and spawning sequence for Guadalupe Bass in either environment. I used behavioral transition probabilities that were greater than 0.15 in order to construct prototypical spawning sequences. The exception to this rule was for behavioral transition probabilities for the sequence contact nip to quiver observed for captive Guadalupe Bass. Although the behavioral transition value was very small (0.005), it was included because it provided the only transition to the sequence for quiver and finally to egg deposition. Another exception was for egg deposition followed by female crossover behavior

(transition probability of 0.10). A similar situation occurred in the wild, where the transition probability between lateral roll and quiver was 0.003. I have included these in the kinematic because they demonstrate that quiver and egg deposition were rare events during the courtship and spawning sequences for Guadalupe Bass in both environments.

3. RESULTS

3.1 Nesting Habitat and Parental Care in Gorman Creek

During June 1 through November 30 of the fall 2012 sampling season a total of 43 Guadalupe Bass were captured by angling in Gorman Creek, Pedernales River, South Llano River, and the Guadalupe River, but none had free flowing gametes. Of these 40 were > 97mm total length, and therefore considered to be adults (Hurst et al. 1975: Edwards 1980). Three fish that were caught escaped during hook removal before length and weight could be measured. No Guadalupe bass nesting sites were observed at any of the four study sites during this study period, and neither courting nor spawning behaviors were observed among Guadalupe Bass.

Between February 2nd and May 10th during 2013 spring sampling, eight Guadalupe Bass nesting sites were observed at Gorman Creek. Six of these eight sites contained fertilized eggs, but three of the sites that had eggs were abandoned for unknown reasons, while the remaining three nests produced Guadalupe Bass that grew into swim-up fry that subsequently dispersed from the nesting site. The majority of active courting and spawning behaviors were observed from March 21 through April 13 2013. Male Guadalupe Bass built four nests on the silt-clay substrate, whereas the remaining four were built on gravel substrate. A fine layer of silt and organic matter covered much of the stream bottom throughout Gorman Creek. Guadalupe Bass males would sweep their caudal fin across the nest until they contacted with the silt-clay gravel bottom. All nests were in pool habitat, and within three meters of permanent in stream structure (Bain 1999), such as a log or boulder. Mean depth of nests was 0.6 m (range of

0.45-0.91 m). Inter-nest distances ranged from 1.2 meters to 100 meters. When nest locations were plotted on a map, two clusters of nesting sites were evident, with one nest site (GC-6) located between the clusters (Figure 2). Nest site GC-6 was 100 m from its nearest neighboring nest site upstream GC-9 and 100 m from its nearing neighboring nest site downstream. The overall mean inter-nest distance was 16.7 m, mean inter-nest distance omitting nest site GC-6 was 4.7 m. Mean nest diameter was 0.49 m (range 0.36 to 0.7 m). Local conditions at nesting sites were included with little to no stream flow, or backwater flow (range -0.02 to 0.02 m/s). Mean water temperature observed during the spring sampling period was 20.2° C (Min: 13.6 and Max: 25.1° C). Nesting habitat data for all nesting sites in Gorman Creek are summarized in Table 2. The habitat and environmental data for each nesting site is summarized in Table 2. Length of parental care varied between the three nesting males that were successful in raising a brood. Length of parental care, date of first observed young, and success of each nesting male are summarized in Table 3.

Parental care was undertaken by the male alone, and varied between 12 to 21 days in length. Males were sculled with their pectoral fins while guarding eggs, also rotated through a full 360° range of orientation while at the center of the nest site. Male Guadalupe Bass remained directly over (within 10 cm of the nest bottom) the nesting site during the two earliest life stages (egg and yolk-sac fry) of Guadalupe Bass young. Once fry became free swimming the male remained in close proximity to the nesting site, but did not hold position directly over the nesting site. I noted that of the three successful nesting sites, all were in areas adjacent to undercut banks, from under which

males could watch the eggs or fry. Guarding males were aggressive towards sunfish, and other smaller Guadalupe Bass that came within close proximity (≤ 1 m) to the males nesting site. Male Guadalupe Bass that successfully produced offspring were relatively large (30 cm TL) as compared to other males in the system. Of the male and female Guadalupe Bass observed in courtship behaviors, none were less than 30 cm TL.

3.1.1 Water Quality Data and Nesting Mat Data (A.E. Wood Hatchery)

Morning temperatures measured in Raceway No. 2 ranged between 16.8 and 19.3° C, mean temperature was 18° C during the morning observation period. Afternoon water temperature ranged between 17 and 19.4 °C, with a mean water temperature of 18.2 °C. Dissolved oxygen (mg/L) measured during the morning ranged from 9.2 to 10.5, with a mean of 9.6 mg/L. Measured afternoon dissolved oxygen ranged from 9.1 and 11.1, with an average of 9.8 mg/L. Nesting mats 2-6, 2-10, 2-14, and 2-18 received eggs on March 14th 2013. Nesting mat 2-2 and 2-18 received eggs again on March 15th 2013. Nesting mats 2-10 and 2-18 had eggs picked up on March 16th 2013. Nesting mats not visible to observations during the study period that received eggs were: 2-11, 2-1, 2-3, 2-11, 2-7, and 2-19. See Table 4.

3.1.2 Physical Characteristics of Guadalupe Bass Fry

I was provided with photographs of Guadalupe Bass eggs and fry by Chris Thibideaux of TPWD to determine their visual differences between eggs and fry from A.E. Wood Hatchery and Gorman Creek. Eggs were clear with an opaque yellow dot in the center of the egg. Eggs were observed at two days in age. The hatched yolk-sac fry were clear in coloration with an opaque yellow yolk-sac in the abdomen and a newly

formed tail. At six days, eyes and the vertebral column were visible and the individual was clear-pearl in color with a dark yolk-sac still attached. At eight days Guadalupe Bass free swimming fry in the hatchery no longer had the yolk-sac and were black in coloration.

Eggs observed in Gorman Creek were clear tan in color with yellow in the center of the egg and were concentrated within the center of the nest. Eggs were adhesive, hatched fry were observed to adhere to the substrate for several days and were blue-pearl in coloration with visible eyes at this stage. Once fry were free swimming, coloration changed to black with a red-orange abdomen. Two to three days before free swimming fry dispersed from the nest coloration changed to clear greenish-brown. Incubation period from egg stage to hatching ranged from approximately three to five days in Gorman Creek. It took approximately three to four days to before fry became free swimming. Fry remained in the nesting site for the following 11 to 14 days, and dispersed after this time period.

3.2 Novel Adult Behaviors: Gorman Creek and A.E. Wood Hatchery

Adult Guadalupe Bass in both environments displayed behaviors not documented in other studies of other Black Basses. Although jaw display behavior, was observed in Guadalupe Bass, and also has been documented in Smallmouth Bass (Ridgway et al. 1989), an additional behavior (opercular flaring), similar but different from jaw display was observed in male Guadalupe Bass in both Gorman Creek and A.E. Wood Hatchery. I describe opercular flaring as the movement of the operculum out and away from the body at angles up to 45°. This behavior was observed primarily in male vs. male

interactions and appeared to be used as a threat display by both individuals during a conflict sequence. It often preceded contact between both individual males. Jaw locking between two males was observed during a 3 m fighting sequence. The males locked jaws and initiated a shoving match over nesting site 2-18. This behavior was observed during this captive video sequence, but this behavior was not observed in Gorman Creek.

Males on nests developed a darker overall body than females, primarily dark olive-green during courting and spawning. The vertical barring of males blended into the darker color of the rest of the body. Females were lighter green in color in contrast to the vertical barring on their sides, which intensified to black during courtship. This made identification of males and females relatively easy during their courting and spawning bouts. Guadalupe Bass males courted individual females as well as multiple females at nesting sites in both Gorman Creek and A.E. Wood Hatchery. This behavior was observed in raceways No. 1 and 2, and raceway No. 4. From one to three females attempted to mate the single male guarding nesting mat 2-18. This multiple female spawning bout was observed on March 13, and eggs were collected by A.E. Wood hatchery staff the next morning. Egg deposition by one of the females, was observed, but certainty of egg deposition by multiple females was not determined.

Multiple females courting with a single male was observed at Gorman Cree for two nesting sites (GC-1, GC-3, and GC-5), on each of the following respective days (4/13/2013, 4/11/2013 and 3/24/2013). During the second courting/spawning event multiple individuals were observed at nesting site GC-1 and I later verified egg deposition by at least one female. Egg deposition was not observed at nesting site GC-5

at a time (3/24/2013) when multiple individuals were using this nest; during observations my presence (in or out of the water) disturbed the females and they stopped courtship activities and left the area. I later verified eggs present at nesting site GC-5. During observations it was difficult to determine if multiple individual females contributed to the eggs observed in the nest at the end of the spawning bout. The male attempted to court all females that were in the nesting site, and all females attempted to interact with the single male. Up to four females were observed to participate in courting with the male at GC-5. Females did not display aggressive behaviors towards other females or the male.

3.3 Length Frequency Data: Fished and Unfished Populations

Twenty-three adult Guadalupe Bass were collected in Gorman Creek throughout the entire study period (fall and spring). Seventeen adult Guadalupe Bass were collected in the Pedernales River throughout the entire (fall and spring) study period (Figure 3). Mean Guadalupe Bass length in Gorman Creek was 285.6 mm TL, and mean weight was 381.1 g, as compared to the Pedernales River, where mean length was 247.3 mm TL and mean weight was 190.4 g. The t-test results indicate that in terms of lengths the Guadalupe Bass from Gorman Creek and Pedernales River are no different (t-Stat = 2.02, p-value = 0.051, DF = 35). In regards to weight the two populations were significantly different from each other (t-stat = 2.25, p-value = 0.03, DF = 29).

3.4 Spatial Distribution of Nesting Mat Use at A.E. Wood Hatchery

Nesting mats 2-10, and 2-18 received eggs on multiple dates during the observation period (two and three dates respectively). Mats 2-2, 2-6, and 2-14 received

eggs once during the observation period. Guadalupe bass males in raceway No. 2 guarded and used nesting mats that were spaced further apart than the actual inter-nest distance between mats as they were provided in both sections the raceway. Guadalupe Bass did not use all of the available nesting mats in proportion to their availability in the raceway (Raceway section 2-1: $\chi^2 = 92.42$, p-value = <0.001, and raceway section 2-2: $\chi^2 = 57.96$, p-value = < 0.001). Nest sites 2-2, 2-6, and 2-10 were used throughout the study period by nesting males in raceway section 2-1. Nest mats 2-14, and 2-18 were used throughout the study period in raceway section 2-2. Guadalupe bass male were using specific nesting mats at a greater frequency to others available within either raceway section (Figure 4).

3.5 Markov Chain Sequence Model

The frequency of observed behaviors for male and female Guadalupe bass was tested against the equiprobable occurrence of behaviors in the full repertoire of observed behaviors. The courtship and spawning behaviors of Guadalupe Bass are not a random assortment of behaviors resulting in successful reproduction ($\chi^2 = 50.69$, p-value = < 0.001, DF = 20). Guadalupe Bass courtship and spawning behaviors have some predictability in their frequency and temporal patterns of behaviors. The analyses indicate that the behavioral sequence of Guadalupe Bass is best fit to a first order Markov chain model (Bakeman and Gottman 1986; Lehner 1996) indicating that the occurrence of a behavior in a sequence is dependent on the preceding behavior observed in sequence.

3.6 Observed Frequencies of Activity States and Behaviors: Gorman Creek and A.E. Wood Hatchery

3.6.1 Observed Frequencies of Activity States

Cumulative observations of activity states differed significantly between both A.E. Wood Hatchery and Gorman Creek during their respective study periods. The conflict activity state was not significantly different in between both A.E. Wood Hatchery and Gorman Creek (z-score -0.65, and z-score -1.07). The sexual activity state differed in their observed frequencies between both environments (z-score -5.14, and z-score 2.95). The sexual activity state was under expressed in Gorman Creek in regards to its expected frequency whereas this activity state was over expressed in A.E. Wood Hatchery. The parental care activity state differed between both environments (z-score 6.84, and z-score -5.57). The parental care activity state was over expressed in Gorman Creek and under expressed by individuals in A.E. Wood Hatchery. This data is summarized in Figure 5 for Gorman Creek and Figure 6 for A.E. Wood Hatchery.

Nesting site GC-1 was compared to nesting mat 2-2 and the binomial z-sore calculated indicated that all three activity states differed significantly (Figure 6). The conflict activity state differed significantly (z-scores -11.15, and 8.64) within both environments (Gorman Creek and A.E. Wood). The conflict activity state was under expressed in Gorman Creek, whereas this activity state was over expressed at A.E. Wood Hatchery. The sexual activity state differed between both environments (z-score 4.09, and -7.86) (Figures 5 and 7). The sexual activity state is over expressed at Gorman Creek, and under expressed at A.E. Wood Hatchery. The parental care activity state did

not differ from its calculated expected value in Gorman Creek (z-score 1.42). At A.E. Wood Hatchery the male differed in the observed frequency, and was under expressed with regards to its calculated expected value (z-score -2.28).

Nesting site GC-1 and mat 2-10 differed significantly in their observed frequency of all three activity states (Figure 5 and 8). The calculated z-scores indicated that the observed frequency of activity state behaviors differed in both environments (z-scores 3.71 and -4.39). The conflict activity state was over expressed in Gorman Creek as compared to 2-10, whereas this activity state was under expressed. Calculated z-scores calculated for the sexual activity state (z-scores -4.83 and 3.60). This indicates that the sexual activity state was under expressed by individuals in Gorman Creek, and over expressed at A.E. Wood Hatchery. Calculated parental care activity state behavior z-scores were (3.73 and -39.91) for each environment respectively. Parental care behaviors were overexpressed at Gorman Creek, and under expressed in individuals at A.E. Wood Hatchery.

Nesting site GC-1 and mat 2-14 differed significantly in their observed frequency of the conflict (z-score -4.91, and 5.16). The conflict activity state was under expressed in individuals in Gorman Creek and over expressed in A.E. Wood Hatchery. The sexual activity states (z-score 3.76, and -7.68) differed from their expected values. The sexual activity state was over expressed in Gorman Creek, whereas this activity state was under expressed at A.E. Wood Hatchery. The frequency of observed parental activity state behaviors differed from the expected value in A.E. Wood Hatchery (z-score 2.43), but was not different from what was expected to have been observed in Gorman Creek (z-

score -1.74). Parental care behaviors were over expressed at A.E. Wood Hatchery. Parental care behaviors in Gorman Creek were observed at a similar frequency to what was expected. See Figures 5 and 9, respectively.

Nesting site GC-1 and mat 2-18 differed in the sexual and parental care activity states. The sexual activity state was under expressed in individuals at Gorman Creek (z-score -5.61). This activity state was over expressed in individuals at A.E. Wood Hatchery (z-score 5.02). Parental care activity state differed from the calculated expected value in both environments. The parental care activity state was over expressed in Gorman Creek (z-score 6.47) and under expressed at A.E. Wood Hatchery (z-score -11.16). There was not a significant difference in the observed frequency of conflict activity state (z-scores 0.58, and -0.65). See Figures 5 and 10, respectively.

Several Guadalupe Bass male and female behaviors differed between the cumulative observed behaviors in both environments (Gorman Creek and A.E. Wood). Three male behaviors: contact nip (z-scores -7.82 and 7.82), circling (z-scores 8.51 and -8.51), and return/sculling (z-scores 5.57 and -5.57) differed significantly between the cumulative observed frequencies of nesting mats in A.E. Wood Hatchery as compared to Gorman Creek (Figures 11 and 13). The contact nip behavior is under expressed in Gorman Creek and over expressed in A.E. Wood Hatchery. Circling was over expressed in Gorman Creek and under expressed in A.E. Wood Hatchery. The return/sculling behavior was over expressed in Gorman Creek whereas it was under expressed in A.E. Wood Hatchery. Four female behaviors: head down (z-scores -13.01 and 13.01), body shudder (z-scores 2.40 and -2.40), lateral roll (z-scores 10.08 and -10.08), and quiver (z-

scores -1.32 and 2.57) differed significantly between the cumulative observed frequencies of nesting mats in A.E. Wood Hatchery compared to Gorman Creek (Figures 12 and 14). The head down behavior was under expressed in Gorman Creek and over expressed in individuals at A.E. Wood Hatchery. Body shuddering was over expressed as compared to its expected value in Gorman Creek, and was under expressed in A.E. Wood Hatchery. The lateral roll behavior was over expressed in Gorman Creek, and under expressed A.E. Wood Hatchery. Quiver did not differ from its expected value in Gorman Creek. It did differ at A.E. Wood Hatchery and was over expressed as compared to its calculated expected value.

3.6.2 Observed Frequencies of Indicator Behaviors

Opercular flaring, escalation/chasing, swim away, contact nip and circling differed significantly between the observed frequency and their expected value in both environments. Opercular flaring occurred at a greater frequency at nesting mat 2-2 than what was recorded for GC-1 (z-scores -2.79 and 2.79). This behavior was under expressed in Gorman Creek and over expressed at A.E. Wood Hatchery. Escalation occurred at a greater frequency at 2-2 than what was observed at nesting site GC-1 (z-scores -4.03 and 4.03). This behavior was also under expressed in Gorman Creek, and is over expressed in A.E. Wood Hatchery. The swim away behavior occurred at a greater frequency at GC-1 than occurred at nesting mat 2-2 (z-scores -3.39 and 3.39). This behavior was under expressed in Gorman Creek and over expressed in A.E. Wood Hatchery as compared to their calculated frequencies. The contact nip behavior occurred at a greater frequency at GC-1 than what was recorded at nesting mat 2-2 (z-scores 2.03

and -2.03). In this case contact nip was over expressed in individuals at Gorman Creek and under expressed in A.E. Wood Hatchery. Circling occurred at a significantly greater frequency at GC-1 than what was recorded at nesting mat 2-2 (z-scores 4.94 and -4.94). This behavior is over expressed at Gorman Creek and under expressed at A.E. Wood Hatchery.

The only female behavior that differed significantly between GC-1 and nesting mat 2-2 was the approach behavior. It occurred at a significantly higher frequency at GC-1 than what was recorded at nesting mat 2-2 (z-scores -2.11 and 2.11). From the z-scores it is apparent that this behavior was under expressed in Gorman Creek and over expressed at A.E. Wood Hatchery. See Figure 13. All other behaviors were did not differ between their observed and expected frequencies in both environments.

Contact nip, circling, and return/sculling differed in the frequencies observed as compared to their calculated expected frequencies. Contact nip occurred at greater frequency at nesting mat 2-10 than what was observed at GC-1 (z-scores -9.26 and 9.26). This behavior was under expressed in individuals at Gorman Creek, and over expressed in A.E. Wood Hatchery. Circling occurred at a greater frequency in Gorman Creek than A.E. Wood (z-scores 5.51 and -5.51). It was also over expressed in Gorman Creek, and under expressed in A.E. Wood Hatchery. Return/sculling occurred at a greater frequency in Gorman Creek than A.E. Wood (z-scores 6.72 and –6.72). This behavior is over expressed in Gorman Creek and under expressed in A.E. Wood Hatchery. The swim away behavior was observed at significantly differing frequencies between Gorman Creek and A.E. Wood and occurred a greater frequency in Gorman Creek (z-scores 4.66

and -4.66). This behavior is over expressed in the male at Gorman Creek and under expressed in the male at nesting mat 2-10. Caudal sweep, and crossover behaviors were also significantly different between both environments (z-scores -2.10 and 2.10) and (z-scores -2.01 and 2.01). Caudal sweeping was under expressed in Gorman Creek with respect to its expected value, and over expressed in A.E. Wood Hatchery. The crossover behavior is under expressed in Gorman Creek, and over expressed at A.E. Wood Hatchery.

Three female behaviors differed significantly in their observed frequencies between nesting site GC-1 and nesting mat 2-10 with regards to their expected values: head down, body shudder, and lateral roll. Head down was observed a significantly greater frequency at nesting site GC-1 than what was recorded for nesting mat 2-10 (z-scores -10.56 and 10.56). Head down was under expressed in the male in the male in Gorman Creek with respect to its calculated expected value. This behavior is over expressed in the males at A.E. Wood Hatchery. Body shudder was observed at a greater frequency at GC-1 than the frequency observed at nesting mat 2-10 (z-scores 2.62 and -2.62). Accordingly this behavior is over expressed in Gorman Creek, and under expressed in A.E. Wood Hatchery. The lateral roll occurred at a significantly greater frequency at GC-1 than what was observed at nesting mat 2-10 (z-scores 5.39 and -5.39). This female behavior is over expressed in Gorman Creek, and under expressed in the female(s) at A.E. Wood Hatchery.

Escalation, bite, caudal sweep, circling, and crossover differed significantly with regards to their expected values. Escalation occurred at a greater frequency at GC-1 than

what was observed at nesting mat 2-14 (z-scores 3.98 and -3.98). The contact nip behavior is over expressed in the male at Gorman Creek, whereas this behavior is under expressed in the male at nesting mat 2-14. The biting behavior occurred at a significantly higher frequency at nesting mat 2-14 and did not occur at nesting site GC-1 (z-scores -8.63 and 8.63). The bite behavior is under expressed in the male at Gorman Creek and is over expressed in the male at nesting mat 2-14. Caudal sweep occurred at a greater frequency at nesting site GC-1 and did not occur during observation at nesting mat 2-14 (z-scores 2.14 and -2.14). The caudal sweeping behavior was over expressed by the male in Gorman Creek and under expressed by the male at nesting mat 2-14. The circling behavior occurred at a significantly greater frequency at GC-1 than what was recorded for nesting mat 2-14 (z-scores 5.46 and -5.46). The male at Gorman Creek over expressed the circling behavior at Gorman Creek, and was under expressed by the male at nesting mat 2-14. The crossover behavior was observed at a significantly greater frequency at nesting mat 2-14 than what was recorded at GC-1 (z-scores -2.82 and 2.82). The male at Gorman Creek under expressed the crossover behavior, whereas this behavior was over expressed by the male occupying nesting mat 2-14.

Female behaviors that differed significantly included: head down, lateral roll, and crossover. The head down behavior was observed at a significantly greater frequency at GC-1 than what was recorded at nesting mat 2-14 (z-scores -6.45 and 6.45). However the head down behavior was under expressed with respect to its calculated expected value. At A.E. Wood hatchery the head down behavior was over expressed in the female courting at nesting mat 2-14. Lateral roll was observed a significantly greater frequency

at GC-1 than what was observed at nesting mat 2-14 (z-scores 4.03 and -4.03). This behavior was over expressed in the female(s) in Gorman Creek and under expressed by the female at nesting mat 2-14. Crossover behavior was recorded at a greater frequency at GC-1 than what was recorded at nesting mat 2-14 (z-scores -2.44, and 2.44). The crossover behavior was under expressed in female(s) at Gorman Creek and was over expressed in the female at nesting mat 2-14.

Contact nip, circling, and return/sculling differed significantly between their observed and calculated expected values. Contact nip occurred at a greater frequency at nesting mat 2-18 than occurred in the mating pair at GC-1 (z-scores -12.16 and 7.91). This behavior is under expressed in the male at Gorman Creek and over expressed by the male occupying nesting mat 2-18. Circling occurred at a greater frequency at nesting site GC-1 than what was observed at nesting mat 2-18 (z-scores 3.18 and -5.86). This behavior is over expressed in the male occupying the nesting site in Gorman Creek, whereas the male occupying nesting mat 2-18 is under expressing the behavior. Return/sculling occurred at a greater frequency at GC-1 and differed significantly from nesting mat 2-18 (z-scores 5.87 and -8.23). The return/sculling behavior was over expressed by the male at the nesting site in Gorman Creek, and under expressed by the male at 2-18. The last male behavior that differed significantly between the observed frequency and the expected frequency was swim away, which was observed at a greater frequency at GC-1 than nesting mat 2-18 (z-scores 3.51 and -4.87). The swim away behavior is over expressed by the male occupying nesting site GC-1, whereas this behavior is under expressed by the male guarding nesting mat 2-18.

Head down, body shudder, lateral roll, and quiver differed significantly between their observed frequencies and calculated expected frequencies at nesting site GC-1 and nesting mat 2-18. Head down behavior was observed at a greater frequency at GC-1 than what was observed at nesting mat 2-18 (z-scores -10.33 and 10.33). The head down behavior is under expressed by the female(s) at GC-1 and over expressed by the female(s) courting at nesting mat 2-18. Body shudder was observed a greater frequency at GC-1 than what was observed at nesting mat 2-18 (z-scores 2.71 and -2.71). The body shudder behavior was over expressed by the female(s) at nesting site GC-1, and under expressed at by the female(s) at nesting mat 2-18. The lateral roll behavior was observed a greater frequency at GC-1 than what was observed at nesting mat 2-18 (z-scores 5.54 and -5.54). The lateral roll behavior was found to be over expressed in the female(s) in Gorman Creek, and is under expressed by the female(s) at nesting mat 2-18. The quiver behavior was observed at a greater frequency at nesting mat 2-18 than it was observed at GC-1 (z-scores -3.23 and 3.23). The quiver behavior was under expressed by female(s) at GC-1, and over expressed in the female(s) at nesting mat 2-18.

3.7 Transition Probabilities and Kinematic Diagrams

Male to male transition probabilities are graphed in (Figure 15) showing all behavioral transitions with a probability greater than 0.20 in Gorman Creek. The greatest transition probability occurred from jaw display to opercular flaring, this value was calculated as a 100% probability that opercular flaring followed the jaw display behavior. This occurred once during observations. Flaring behavior was followed by swim away with a transition probability of 0.39. Opercular flaring was preceded by

escalation behavior in the male with a transition probability of 0.67. Sculling behavior was the preceding behavior to chasing in male Guadalupe Bass at nesting site GC-1, and this transition probability was 0.81. Sculling behavior was the preceding behavior to swim away with a calculated transition probability of 0.93. Sculling with rotation followed sculling behavior with a transition probability of 0.47, while sculling with rotation preceded sculling with a transition probability of 0.42.

Male behavioral transitions in A.E. Wood were more complex than in Gorman Creek (Figure 16). More behaviors were involved that had significant transition probability values (≥ 0.20). Opercular flaring preceded escalation with a transition probability of 0.37. Escalation and chasing preceded sculling with rotation with a probability of 0.20, and sculling with rotation followed itself with a probability of 0.46. Escalation and chasing preceded sculling with a transition probability of 0.26. Swim away followed the sculling behavior with a transition probability of 0.32, and swim away also preceded return and sculling with a transition probability of 0.67. Biting behavior preceded escalation, flaring, sculling, and caudal sweeping with respective transition probabilities of 0.40, 0.20, 0.20, and 0.20. Circling behavior preceded contact nip behavior with transition probabilities of 0.48, and contact nip preceded itself with a transition probability of 0.50.

Female to female interactions in Gorman Creek are graphed in Figure 17. Female behaviors were limited to nine indictor behaviors, as listed in the ethogram. Female body shuddering followed the approach behavior with a transition probability of 0.46. Following head-down behavior was observed to follow body shuddering with a

transition probability of 0.18. Lateral roll behavior followed head-down with a transition probability of 0.48. Lateral roll followed itself with a transition probability of 0.35. Lateral roll also follows body shuddering with a transition probability of 0.16, body shuddering followed itself with a transition probability of 0.18 during observation. Circling behavior followed lateral roll with a transition probability of 0.15, and lateral roll followed circling behavior with a transition probability of 0.71. Lateral followed crossover with a transition probability of 0.43, and body shudder followed crossover with a transition probability of 0.17. Quiver behavior followed lateral row with a probability of 0.003, egg deposition followed quiver with a transition probability of 1.0.

Female to female interactions for individuals at A.E. Wood are graphed in Figure 18. Crossover behavior followed approach with a transitional probability of 0.17. Head down behavior followed crossover behavior with a transition probability of 0.19, and head down behavior followed rise and lateral row with transitional probabilities of 0.67, and 0.17. Lateral roll behavior followed itself with a transition probability of 0.17 during observations. Head down behavior followed circling behavior with a transitional probability of 0.27 during observations. Body shudder followed swim away behavior with a transition probability of 0.22. Egg deposition followed quiver with a transition probability of 0.17. Circling and swim away behavior followed egg deposition with transition probabilities of 0.25 for each transition.

3.8 Prototypical Courtship and Spawning Sequence in Guadalupe Bass

A prototypical courting and spawning sequence of Guadalupe Bass in Gorman Creek involved three male behaviors: contact nip, caudal sweep, and circling. Seven

female behaviors: approach, head down, body shudder, lateral roll, circling, quiver, and egg deposition (Figure 19). Female Guadalupe Bass typically started the sequence by approaching the male and the nesting site. Contact nip and body shuddering followed the approach behavior. Transition probabilities were 0.15, and 0.46 respectively. Contact nip and body shuddering followed caudal sweeping with transition probabilities of 0.27, and 0.45. Circling, lateral roll, and body shudder followed contact nip with transition probabilities of 0.05, 0.23, and 0.39, respectively during observation. Head down followed body shudder with a calculated transition probability of 0.18. Lateral roll followed head down position with a transition probability of 0.48, and head down position followed lateral roll with a transition probability of 0.12. Lateral roll followed circling with a transition probability of 0.70, circling followed lateral roll with a transition probability of 0.15. Female circling followed lateral roll, and body shudder with transition probabilities of 0.15 and 0.13, while lateral roll, and body shudder followed female circling with transition probabilities of 0.71 and 0.13. Quiver followed lateral roll behavior at a transition probability of 0.003, egg deposition followed quiver with a transition probability of 1.0 during observation.

The prototypical courting and spawning sequence for Guadalupe Bass at A.E. Wood was composed of four male behaviors: contact nip, caudal sweep, circling, and crossover. Eight female behaviors: approach, head down, body shudder, lateral roll, circling, crossover, quiver, and egg deposition (Figure 20). Females approached and was followed by contact nip in the male with a transition probability of 0.50. Male crossover and female crossover followed the approach behavior with transition probabilities of

0.17, and 0.17 respectively. Male circling and head down followed contact nip with transition probabilities of 0.05, and 0.26. Head down position followed male crossover and male circling with calculated transition probabilities of 0.25, and 0.23. Head down, body shudder, and lateral roll were followed by caudal sweeping with respective transition probabilities of 0.19, 0.22, and 0.11. Head down was followed by lateral roll with a transition probability of 0.17. Contact nip and head down followed female circling with transition probabilities of 0.50, and 0.27 respectively. Quiver followed contact nip at a transition probability of 0.005, egg deposition followed quiver with a transition probability of 0.17, and egg deposition also followed female crossover with a transition probability of 0.10. Egg deposition was followed by female circling at a transition probability of 0.25.

4. DISCUSSION

The reproductive life history of the Guadalupe Bass was examined in my study, and is the first to publish detailed descriptions of the courting, spawning, and parental care behaviors of this unique Texas native. It appears that there are several mechanisms that may contribute to the initial and continued persistence of hybrid Guadalupe Bass x Smallmouth Bass hybrids. After the completion of this study, I am left with far more questions than answers regarding the reproductive life history of the Guadalupe Bass. The new information regarding the nesting habitat used, spacing of nesting sites, length of parental care, and observations of the early life stages of the Guadalupe Bass have been described and documented for the first time in detail in my study. The observation of multiple individuals courting simultaneously begs the question as to why this behavior occurs. Female copying, alternative mating tactics, and lack of available nesting sites may be possible explanations for the observed behavior. These alternative hypotheses will be discussed and explored further throughout this discussion.

4.1 Nesting Habitat (Gorman Creek) and Parental Care

Guadalupe Bass use similar nesting habitat, and exhibit parental care, courting, and spawning behaviors similar to what is described for other Black Bass species (Ridgway et al. 1989; Winemiller and Taylor 1982; Williams and Burgess 1999; DeWoody et al. 2000). Nests were constructed during mid-March through early April in Gorman Creek. Nesting sites were recycled by the original male or by a new male. This occurred on several occasions during the course of the study. Guadalupe Bass built nests in pool habitat, at depths up to 1 m, with very little flow (Table 2). Guadalupe Bass in

Gorman Creek built nesting sites in close proximity to permanent structure and in the case of the three successful nesting sites in Gorman Creek (GC-5, GC-6, and GC-7) were built within 1 meter of an undercut bank. The males at other nesting sites were not successful in raising their broods (Table 3). Guadalupe Bass built nesting sites near permanent structure similar to what has been documented for Largemouth and Smallmouth Bass (DeWoody et al. 2000; Hunt and Annett 2002; Hunt et al. 2002; Winemiller and Taylor 1982). These nesting sites were near permanent structure but not within close proximity to an undercut bank. The guarding male at nesting site GC-7 built his nest entirely underneath an undercut bank. This provides adequate protection for the guarding male from avian predator and human predators (Hunt et al. 2002). I observed Great Blue Herons *Ardea herodias* wading and hunting at Gorman Creek. The other advantage to the guarding male selecting a nesting site near a dark undercut bank provides shade to enhance vision (Helfman 1981). A shaded Guadalupe Bass should be able to see a sunlit target (i.e. a potential nest predator) at more than 2.5 times the distance than a sunlit potential nest predator would be able to see the shaded guarding Guadalupe Bass (Helfman 1981).

Suitable nesting habitat may be a limiting factor to the number of nesting males in Gorman Creek. Gorman Creek has extensive overgrown stretches of cow lily pads *Nuphar luteum*, which provides cover for nest robbing sunfish species. In a study of the behavioral and habitat selection of nesting Largemouth Bass males, brood predators were most abundant in complex physical structure (Hunt et al. 2002). Parental males were guarding nesting sites within close proximity to complex structure experienced

intrusion rates four to seven times higher than males whose nesting sites were within close proximity to less complex structure (Hunt et al. 2002). Males that nested within close proximity to complex structure displayed higher rates of aggression as well. Guadalupe Bass nesting sites were not found within close proximity to lily pad patches. If a male Guadalupe Bass cannot see an approaching nest intruder in complex structure then the male will be ineffective at guarding his brood. It has been documented in Largemouth Bass that as habitat complexity increases the effectiveness of hunting bluegills decreases (Savino and Stein 1982).

Guadalupe Bass would face the same problem in a complex habitat similar to the overgrown lily pads that are present in stretches of Gorman Creek. This could reduce suitable nesting habitat available to male Guadalupe Bass and result in a relatively small number of dominant males that secure suitable nesting sites. Guadalupe Bass exhibit resource defense polygyny in regards to their mating system males control access to females indirectly, through the monopolization of critical resources, such as a suitable nesting site (Emlen and Oring 1977). Larger, dominant males may be able to attain suitable nesting sites, and may be able to court multiple females during the spawning season, thus increasing that male's contribution to the next generation.

Male Guadalupe Bass guarded their nesting sites and broods without the aid of the female Guadalupe Bass. It appears that in this aspect of parental care Guadalupe Bass are typical in this characteristic to the rest of the *Micropterus* genus. Guadalupe Bass were observed to scull within the nesting site while rotating. The sculling behavior is thought to facilitate adequate oxygen flow over the fertilized eggs. The rotation

behavior likely facilitates early detection of nest intruders (Winemiller and Taylor 1982). This behavior has been observed in Smallmouth Bass in Indian Creek, Ohio (Winemiller and Taylor 1982). Longear Sunfish, and Green Sunfish, and red swamp crayfish *Procambarus clarkii* occur in Gorman Creek and these three species are more than capable of making quick meals of unguarded Guadalupe Bass eggs.

4.1.1 Novel Behaviors in Guadalupe Bass and Implications for Mate Choice Studies

Guadalupe Bass exhibited several unique behaviors during the course of my study. Male Guadalupe Bass were observed to use opercular flaring during male confrontations and appeared to be a threat display. A similar behavior occurs in male Siamese fighting fish *Betta splendens.* In this species it has been determined that this behavior is used as a means to intimidate an opponent (Allen and Nicoletto 1997). This behavior only occurred in Guadalupe Bass during male vs. male interactions, and was associated with conflict behaviors. Typically males would orient facing each other, and would flare the operculum perpendicular to the body at angles up to approximately 45°. This behavior has not been reported anywhere else in the published literature for Black Basses. A similar behavior (jaw display) has been observed in Smallmouth Bass (Ridgway et al. 1989), and was observed in Guadalupe Bass as well during the course of my study. The jaw display behavior which is described as the male partially opening the mouth and spreading the branchiostegal membrane to accentuate the outline of the lower jaw (Ridgway et al. 1989). This behavior was observed in my study and as such I am quite confident that jaw display and opercular flaring are two distinct behaviors.

Male fighting was observed in both environments (A.E. Wood Hatchery and Gorman Creek) although to a greater extent in the hatchery setting. A three minute focal video involved two males fighting and jaw locking near nesting mat 2-18. Fighting appeared to be over the nesting mat or a female that the resident male at 2-18 was courting. Jaw locking was a new behavior that has not been reported for any of the Black Basses in the literature. Once Guadalupe Bass were in the jaw locked position the males then began a shoving match. This shoving match ended once the resident male had pushed the intruding male to the other side of the raceway compartment and away from the resident male's territory. Male fighting in Gorman Creek was limited to quick opercular flaring, escalation involving chasing towards an intruding male by the resident male at GC-1. Fighting was not observed at Gorman Creek and is likely because males were able to spatially distribute themselves from other male's nesting sites.

Guadalupe Bass males and females were observed to change coloration during courting and spawning bouts. Males' bodies darkened intensely to a dark-green color, the vertical barring becoming very faint. In females the vertical barring pattern along the sides of their bodies darkened to black in coloration, while the rest of the body became light green in color. Males and females were easily identifiable during courting and spawning bouts. This has been documented in other *Micropterus* species (Breder and Rosen 1966; Heidinger 1975; Miller 1975; Trautman 1981; Williams and Burgess 1999). In females of the genus *Lepomis*, this is believed to act as a submissive signal to the male. Guadalupe Bass males look similar to Smallmouth Bass males during courting bouts. This change in color pattern may be an avenue of miscommunication between

Guadalupe Bass and Smallmouth Bass during the spawning season. It has been observed in darters that color pattern is used as a means of communication between species to ensure correct mate choice (Williams and Mendelson 2011). This method of behavioral isolation would work well in systems where two closely related species have co-evolved. Female Splendid Darters *Etheostoma barrenese* and female Banded Darters *Etheostoma zonale* were observed to select males of their respective species based on color pattern alone (Williams and Mendelson 2011). Is male and female coloration an important factor in mate choice selection in Guadalupe Bass? In Smallmouth Bass total length and body stoutness of males were the only reliable indicators of female choice (Hanson and Cooke 2009). Other studies suggest that male body size have some effect on mate choice and success while guarding eggs and fry (Wiegmann and Baylis 1995; Wiegmann et al. 1997). These predictors of mate choice could be selection factors in female Guadalupe Bass. This would provide another vector of error in selection of Smallmouth Bass mates rather than selecting the "correct" Guadalupe Bass male.

If color pattern is irrelevant in female mate choice, but larger male body size is relevant, then large males of Smallmouth Bass should be chosen by female Guadalupe Bass over most Guadalupe Bass males where they co-occur, because male Guadalupe Bass are typically smaller in size than male Smallmouth Bass (Warren 2009). Female Guadalupe Bass may be making the best choice for the survival of their young, and thus their own genetic fitness, but making the wrong choice in regards to the genetic integrity of the Guadalupe Bass population. These factors may contribute to the continued

persistence of hybrid forms and the decline of pure strain Guadalupe Bass across its native distribution in central Texas.

Male Smallmouth Bass would have a significant advantage over Guadalupe Bass as Smallmouth Bass naturally attain a larger mean size (Warren 2009). Future studies should focus on answering whether coloration differences between males and females or total length and body stoutness are reliable predictors of mate choice in Guadalupe Bass. Mate choice studies that examine selection of males by female Guadalupe Bass in a captive environment similar to what was done in Williams and Mendelson (2011) to examine the importance of male coloration in female mate choice.

4.2 Simultaneous Participation of Multiple Individuals in Captivity and Wild Environment

It has been recognized that male Black Bass' will attempt to mate with multiple females during the spawning season (Heidinger 1975; Isaac et al. 1998; Wiegmann et al. 1992). However it was not certain whether this is done sequentially or simultaneously in other Black Bass species. Guadalupe Bass males are the first observed to court with multiple (2+) females simultaneously. What is the purpose behind this novel behavior? Two alternate hypotheses to explain the purpose of this behavior are female copying, or parasitic mating tactics. There is some evidence from my study that supports both hypotheses and they will be discussed in further detail.

Female copying behavior may be at present in female Guadalupe Bass. Mate copying was found to have a role in mate choice in the Trinidadian Guppy *Poecilia reticulata* (Dugatkin 1992). Female Guadalupe Bass may be selecting male Guadalupe

Bass based on whether another female is currently mating with that male. The second female would have based her decision simply on the fact that another female was already courting with the male. Female copying benefits the female through information sharing which could reduce the time spent searching for a mate, as well as quickly finding a high quality mate. Female copying is advantageous in situations where resources (i.e. nesting sites/males) are distributed in a patchy manner. Female groups would then become distributed in a patchy distribution as well (Shuster and Wade 2003). Nesting sites may be a limiting resource in Gorman Creek. The aggregation of females at a resource (nesting site) should increase the variance of male mating success (Shuster and Wade 2003). This is concurrent with what was observed in Gorman Creek.

Three other fish species have evidence supporting female copying. The River Bullhead *Cottus gobio*, Fathead Minnow *Pimephales promelas,* and Fantail Darter *Etheostoma flabellare* (Pruett-Jones 1992; Dugatkin 1992; Marconato and Bisazza 1986; Unger and Sargent 1988; Knapp and Sargent 1989). In the case of the River Bullhead females were more likely to deposit eggs in a male's nest that already had eggs in the nest from a previous female. In the Fathead Minnow females preferred to spawn with males that already had eggs in their nest (Unger and Sargent 1988). Female Fantail Darters were found to prefer males that already had eggs within their nesting site (Knapp and Sargent 1989). In these previously published studies females were found to mimic the mate choice of other females that had previously mated and deposited eggs with a male holding a nesting site. Although these studies specifically focus on the presence of absence of eggs within the nesting site, females may associate the presence of eggs with

a high quality male. During captive observation of Guadalupe Bass female individuals were not able to see eggs that were already in the nesting mat. Nesting mats that had eggs were quickly removed from the raceways at A.E. Wood in the morning when they were found. During the observations of multiple individuals courting at Gorman Creek (GC-1) eggs were not within the nesting sites previous to multiple females courting with the resident guarding male. Female Guadalupe Bass may be interpreting information in regards to the quality of male Guadalupe Bass and their potential for protecting a brood successfully. A male that has, and can hold a nesting site, and can attract one female to his nest is a desirable mate.

Female Smallmouth Bass were found to select mates who were large (length and stoutness). Hanson and Cooke (2009) found that male total length and body stoutness were reliable predictors of female male choice. It is not known whether Guadalupe Bass females use this decision process in choosing mates. Female Guadalupe Bass may use a combination of body size, and female copying behavior in selecting a male Guadalupe Bass. At this time the decision making process in female Guadalupe Bass for selecting a male mate is not well understood. I propose that female selection of mates should be further examined in the Guadalupe Bass to better understand why multiple individuals were observed to court simultaneously.

These behaviors may not be a mutually exclusive event to Guadalupe Bass. Detailed field observations of Black Bass courtship and spawning are limited for several reasons. Spawning can last for several hours (~ 4 hours at GC-1), and the majority of activity takes place during low light conditions (Cooke et al. 2003). Multiple individuals

courting in a single nesting site may be more common within the Black Basses. It may simply have not been observed yet, or occurs within certain populations and not others. The other range restricted Black Basses are not well studied and have little information published in regards to their courtship and spawning behavior. The typical descriptions of courting, spawning, parental care has been identified for the Black Basses and are primarily based on the Largemouth Bass, Smallmouth Bass, and Florida Bass (DeWoody et al. 2000; Ridgway 1989; Winemiller and Taylor 1982). A typical courting event involves the male approaching and guiding a female to the nesting site. This is followed by behaviors similar to what has been observed in this study of Guadalupe Bass. Males are reported as courting with a single female, and may attract and spawn with multiple mates but at different times during the spawning season. However this typical courting sequence appears to have been applied to the rest of the species within the Black Bass group. It is assumed that a successful pair's brood consists of young that are sired by a single female and the guarding male. Female copying is an alternate hypothesis and would be expected in a system where resources (nesting sites) are limited within the environment. Experimental trials are need to better understand the purpose of females aggregating at nesting sites with a single male Guadalupe Bass. Future studies into the purpose of multiple individuals courting simultaneously in Guadalupe Bass should be further investigated as well as determine the actual parental contribution of fertilized eggs in Guadalupe Bass nests where multiple individuals are courting.

4.3 Presence of Alternative Reproductive Tactics in Guadalupe Bass?

The observation of multiple individuals attempting to court and spawn raises questions as to why this is occurring within this species. One such hypothesis to investigate in future studies of the Guadalupe Bass are alternative mating tactics in males. Alternative mating tactics are observed in the closely related genus *Lepomis*. Alternative mating tactics include sneaker and satellite males, broadly categorized as a "parasitic" tactics (Taborsky 1997). Sneaker males are individuals that use quick nest entry and exit during spawning of a parental male and female individuals. Satellite males use a slower approach into the nest site, and are of relatively equal size to a parental male. These satellite individuals have a body color similar to that of a female (Neff and Knapp 2009). Bluegill *Lepomis macrochirus,* Longear sunfish *Lepomis megalotis,* Pumpkinseed *Lepomis gibbosus,* and Spotted Sunfish *Lepomis punctatus* have been documented to have a parasitic morph in the natural environment (Neff and Knapp 2009).

Although these alternative mating tactics have not been documented in *Micropterus* it would be unwise to make the assumption that this does not occur. It has been determined with some degree of certainty that Largemouth Bass and Smallmouth Bass lack a parasitic morph (DeWoody et al. 2000; Gross and Kapuscinski 1994). The lack of information regarding the reproductive biology, and behavior of Guadalupe Bass prior to this study prevents me from concluding that Guadalupe Bass exhibit or lack a parasitic male morph. Further investigation into my observations of multiple individuals courting simultaneously should reveal whether parasitic morphs occur within the

species. Three additional species within the family *Centrarchidae* have been identified as lacking parasitic morphs: Dollar Sunfish *Lepomis marginatus*, Redbreast Sunfish *Lepomis auritus*, and Rock Bass *Ambloplites rupestris*. For the remaining 23 species within the family there is not enough data to conclude whether a parasitic morph exists in these species (Neff and Knapp 2009). There is a possibility that some of the female individuals observed to take part in courting were not females but male female-mimics taking advantage of a parental male. As this behavior (multiple courting individuals) was not anticipated prior to the start of this study there was no effort to account for identifying the sex of individuals participating in this behavior. Coloration was used to identify males and females as well as by the behaviors that each fish was expressing. It was found that in Bluegills the female mimics copied female behavior but were much smaller than the resident nesting male Bluegill (Dominey 1980). However all individuals that were described and identified as females displayed female behaviors, and were of similar size (≥ 30 cm) to the resident male. I was not able to determine which individuals contributed gametes to the eggs deposited in A.E. Wood Hatchery and Gorman Creek. Future studies of the reproductive behaviors of the Guadalupe Bass should focus on documenting the presence or absence of parasitic male life history in the Guadalupe Bass.

4.4 Nesting Mat Use in A.E. Wood Hatchery

Micropterus males are identified as solitary nesters, and establish a territory well-spaced from other neighboring males (Warren 2009). In the captive setting (A.E. Wood Hatchery) Guadalupe Bass were observed to use specific nesting mats during

observation. In theory all nesting mats are identical. Each nesting mat is of the same design, material, and manufacturer, as well as the same dimensions (45 × 60 cm). Largemouth Bass kept and spawned at A.E. Wood Hatchery and East Texas State Hatchery have been observed to use every available nesting site provided to them regardless of the inter-mat distance (C. Thibideaux and R. Sparrow, Texas Parks and Wildlife Department, personal communication 2013).

The results of the Chi-square goodness of fit test indicated that Guadalupe Bass males were using only specific nesting mats in raceway No. 2. The frequency of Guadalupe Bass observed at each nesting site was greater or less than what would be expected to have been observed with an equiprobable distribution. We assume that because all nesting mats are equal there should be no preferential use of nesting mats by male Guadalupe Bass. Nesting mats 2-2, 2-6, 2-10, 2-14, and 2-18 were used extensively by male Guadalupe Bass and received eggs during the observation period. Nesting mats 2-2 and 2-10 are several meters apart from each other in section 2-1. Nesting mats 2-14 and 2-18 were several meters apart from each other as well. Guadalupe Bass males appear to be selecting nesting mats greater than 1.5 m of internest distance, which is the distance afforded between adjacent nesting mats. Average internest distance measured in Gorman Creek was 16.7 m. This value is likely inflated because of nesting site GC-6, which was 100 m from its nearest neighbor downstream and upstream of its location. When this nesting site is not included in the calculation of the average internest distance, average internest distance is 4.74 m in Gorman Creek. This internest distance is greater than the distance between nesting mats at A.E. Wood Hatchery.

When looking at all available nesting mats in raceway No. 2 including those that were not visible from the observation platform it would appear that Guadalupe Bass males are attempting to maximize the inter-mat distance. Nesting mats 2-1, 2-3, 2-7, and 2-11 received eggs during the observation period. With the exception of 2-1 and 2-3 the other two nesting mats have an empty nest as their nearest neighbor. These nests are also not directly across from nesting mats that received eggs (2-6 and 2-10). Within section 2-2 of raceway No. 2 nesting mat 2-19 received eggs and was not directly across 2-18 and was the only other nesting mat to receive eggs besides nesting mats 2-14 and 2-18. Guadalupe Bass at A.E. Wood Hatchery appear to nest at distances greater than 1.5 m as is afforded to them in the hatchery setting.

Indirect evidence (Lutz-Carrillo et al. 2013) supports the conclusion that a relatively small proportion of the male population of Guadalupe Bass within each raceway section is securing access to the majority of females within the raceways. This could have a direct effect on the effective population size of the fry that are released back into the wild for restoration efforts. The results of my study are consistent with previously collected and analyzed genetic data (Lutz-Carrillo et al. 2013). Guadalupe Bass are selecting and using specific nesting mats continuously during the duration of spawning in A.E. Wood Hatchery.

Measures to ensure that effective population size remain high in the hatchery setting would be beneficial to continued restoration efforts of Guadalupe Bass. Several possible actions include: reducing the total number of Guadalupe Bass placed in each raceway section, placing structures to provide a barrier between nesting mats that will

allow a greater proportion of males to participate in mating, and dividing the raceways into smaller sections and placing fewer Guadalupe Bass per section to ensure an equal distribution of mating opportunities.

4.5 Activity State Frequencies and Behavioral Frequencies of Hatchery and Natural Guadalupe Bass

Guadalupe Bass exhibited differing frequencies of activity states between both environments (A.E. Wood Hatchery and Gorman Creek). Activity states differed between the captive and wild environments in the sexual and parental activity states. There was no significant difference in the observed frequencies of the conflict activity state between the two environments. The sexual activity state was observed at a greater frequency in A.E. Wood Hatchery and over expressed by males in this environment, whereas this activity state was under expressed by the male observed in Gorman Creek. The parental care activity was over expressed by the male observed in Gorman Creek, yet under expressed by males in A.E. Wood Hatchery. The most likely cause for this is the daily removal of newly laid eggs at each of the males nesting mats in the captive environment. Males were not able to provide parental care to their young and were thus free to actively pursue additional females to court and spawn. This is evident when we examine the corresponding z-scores for the sexual and parental care activity states from both environments.

The two environments are inverse in the activity states that the male(s) expressed during observation. The male Guadalupe Bass in the hatchery expressed more sexual activities because they had “lost” eggs and were free to continue courting, whereas the

male in Gorman Creek received eggs and spent a substantial amount of time guarding those eggs. This male's time was skewed towards defense of his current clutch of eggs, and was not free to actively pursue or attract new females.

The observed frequencies revealed significant differences in the observed frequency of behaviors between both environments (A.E. Wood Hatchery and Gorman Creek). Male behaviors differed significantly in the following behaviors: contact nip, circling, and return/sculling. The contact nip behavior was under expressed in the male in Gorman Creek and was over expressed by males in A.E. Wood Hatchery. The circling behavior was over expressed in Gorman Creek and under expressed in individuals at A.E. Wood Hatchery. Return/sculling was over expressed in the male occupying the nesting site in Gorman Creek, and is under expressed at A.E. Wood Hatchery. Males were not likely to spend time sculling in the hatchery as eggs were pulled from the nesting mat within 24 hours. The male Guadalupe Bass in Gorman Creek was observed to guard his nesting site (and eggs) for a large portion of the day.

When examining the cumulative observed frequencies of all behaviors in captivity to nesting site GC-1 there was three female behaviors which differed significantly between the two environments: head down, body shudder and lateral roll. Females in Gorman Creek were more likely to over express the body shuddering and lateral rolling behaviors. Females in A.E. Wood Hatchery over expressed the head down behavior. These three behaviors represented the core set of female behaviors in the prototypical kinematic diagrams for both environments. The quiver behavior was a rarely observed behavior and was expressed at a greater frequency in A.E. Wood

Hatchery than what was observed in Gorman Creek. This is likely because of the removal of eggs from nesting mats. As discussed earlier in this section, males with no eggs to guard would seek out additional females to court. The male in Gorman Creek maintained his eggs for a short time and was not observed to pursue additional mating opportunities.

4.6 Predictability of Behavioral Sequences and Kinematic Diagrams

4.6.1 Markov Chain Model Type

The results of the Chi-square goodness of fit test were used to determine what type of Markov chain model to follow indicated with significance ($\chi^2 = 50.69$, p-value = < 0.001, DF = 20) that the observed frequencies of behavior followed a first order Markov chain model. The p-value indicates that the observed frequencies of Guadalupe Bass behaviors are significantly different from random frequencies. This indicates that the probability of a behavior occurring is dependent upon the observed behavior that preceded it. The primary purpose of this test was to determine which model to follow for further analysis of the observed frequency of behaviors, and the sequence order of behaviors. Guadalupe Bass behaviors during courting and spawning have predictability in their progression from initiation of courtship to egg deposition and fertilization. The male to male behavioral sequences differed between the two environments, in both the behaviors involved and the significance of the transition probabilities between those behaviors. Six observed behaviors were used to construct the kinematic diagram of male to male behaviors of the individual in Gorman Creek (Figure 15). Nine observed

behaviors were used to construct the kinematic diagram for male to male kinematic diagram of the male to male behaviors of the individuals in A.E. Wood Hatchery.

4.6.2 Kinematic Diagrams and Transition Probabilities

Three conflict activity state behaviors comprised the behaviors used to construct the kinematic diagram. Two parental care activity state behaviors were used in addition to the three conflict behaviors. The swim away behavior was used to complete the kinematic diagram. These kinematic diagrams illustrate the sequence of behaviors through time that the male Guadalupe Bass exhibited with regard to his previous and following behaviors. In the captive setting (A.E. Wood Hatchery) involved a greater set of behaviors with significant transitions (Figure 16). Three sexual activity state behaviors were used to construct the kinematic diagram. Three conflict activity state behaviors were used to construct

The female to female kinematic diagram (Figure 17) was constructed using eight female behaviors for the individuals observed at Gorman Creek (GC-1). Female to female kinematic diagram was constructed using 10 behaviors observed in females at the A.E. Wood Hatchery (Figure 18). Behaviors used were similar for females in both environments, with the exception of the rise behavior. This behavior was not observed in any of the females observed in the wild. These kinematic diagrams simply demonstrate the sequence of behaviors within each sex during courtship, spawning and parental care activity states. We are able to examine the differences in the sequence, transition probabilities and the behaviors involved of the two sexes between the two environments used in this study. On their own these kinematic graphs do not properly convey the

sequence of reproduction in the Guadalupe Bass. The next section discusses in detail the constructed prototypical courting and spawning sequence of the Guadalupe Bass, in the captive and wild environments.

4.7 Prototypical Courtship and Spawning Sequences

The kinematic diagrams (Figure 19; Figure 20) of the prototypical courtship and spawning sequence of Guadalupe Bass differed between the two environments (A.E. Wood Hatchery and Gorman Creek). Despite the differences between the transition probabilities, the sequence of the courting and spawning behaviors follow a similar pattern amongst the individuals (male and female) from both environments. In Gorman Creek the female would approach the male while the male was situated over the nesting site. The male would then react with a contact nip at the females body (usually the mouth, operculum, abdomen or the vent) upon her approach and entry into the nesting mat/site. This was followed by the female body shuddering or moving into the head down position behavior. Circling behavior in the male usually followed contact nip, although at a very low transition probability (0.05). Caudal sweeping preceded contact nip and body shuddering in the female. During observation sequences of behaviors often repeated during a courting sequence. The lateral roll behavior followed itself often during observation. Lateral rolling is not a continuous behavior, and is a discrete set of movements. Circling followed the lateral roll and body shuddering behaviors, and in turn after circling behavior the female returned to lateral rolling, and body shuddering. The lateral rolling preceded the observation of quivering behavior. The quiver behavior in females was always followed by egg deposition when observed.

It was evident that a core set of female behaviors dominated the typical spawning sequence of Guadalupe Bass in Gorman Creek. The head down, body shudder, and lateral roll behaviors had significant transition probabilities with most of the other behaviors used in the kinematic diagram. Three male behaviors had significant transitions to the core set of female behaviors (Figure 19). Males used contact nips to attempt to get females to deposit eggs. There is one sequence where this resulted in successful deposition of eggs within the nest. The quiver behavior occurred once during observation in Gorman Creek and the transition probability is very small (0.003). Females appeared to get stuck in a "feedback loop" where no matter what the male's preceding behavior was (i.e. contact nip, caudal sweep, or circling etc.) the female would not deposit eggs. The male had to continually tend to the female before the female would eventually deposit eggs for the male to fertilize.

The typical courtship and spawning sequence observed in A.E. Wood Hatchery (Figure 20 followed a similar although varying order of sequence to what was observed in Gorman Creek. Females would approach the male at the nesting mat. Males occasionally sought out females in the hatchery environment. It did not occur at a very high frequency in either environment. This behavior was followed by a contact nip from the male. Male and female crossover behavior also followed the female's approach behavior during observation. Females typically responded to the contact nip by positioning themselves head down within the nesting mat. Male crossover behavior and circling behavior preceded the head down position in females. The caudal sweeping behavior preceded head down position, body shudder, and lateral roll. Head down

behavior follows the lateral roll behavior. There were two behaviors that preceded the deposition of eggs in the captive setting. Quiver, and female crossover immediately preceded egg deposition. Female circling followed immediately after the deposition of eggs. The circling behavior was followed with a high probability of receiving a contact nip from the male, and an intermediate probability of the head down position following female circling. This was a typical courtship and spawning sequence that occurred during the observations of courting in A.E. Wood Hatchery.

The prototypical courting and spawning sequence in A.E. Wood Hatchery followed a similar sequence to what was observed in Gorman Creek. A core set of female behaviors dominated the kinematic diagram (Figure 20). The females exhibited the same "feedback loop" set of behaviors (head down, body shudder, and lateral roll) as was observed in Gorman Creek. The males used the same behaviors (caudal sweep, contact nip, circling, and crossover) to instigate the female into depositing eggs. In the A.E. Wood Hatchery an instance occurred where a direct contact nip behavior resulted directly in egg deposition, however this was observed once and the transition probability reflects this (0.005).

These descriptions of the prototypical courtship and spawning sequences of Guadalupe Bass observed in A.E. Wood Hatchery and Gorman Creek are by no means a set-in-stone description of the sequence that Guadalupe Bass follows across its entire distribution. Instead it merely provides the first attempts to document and describe the general pattern of behaviors that took place during observations. There is variation visible between the two constructed prototypical courting sequences of individuals

between the two environments. Variation is inherent between individuals within and across environments, and it is important to stress that these kinematic diagrams are representative of the general patterns of behavioral sequences during courtship and spawning in this species.

5. CONCLUSIONS

5.1 Conclusions

Nesting habitat used by Guadalupe Bass is similar to the nesting habitat used by Smallmouth Bass in lotic systems (Bozek et al. 2002). Spatially, Guadalupe Bass and Smallmouth Bass are likely to use similar habitat to construct nests. Large Smallmouth Bass may displace male Guadalupe Bass from suitable nesting sites throughout stream and rivers where Smallmouth Bass have been introduced. It has been determined that that niche partitioning occurs in *Micropterus* species that historically overlapped in distributions (Wheeler and Allen 2003; Goclowski et al. 2013). Despite reintroduction efforts, Smallmouth Bass persist in these systems and will continue to contribute to recruitment of hybrids.

Guadalupe Bass and Smallmouth Bass are similar in the behaviors expressed during courtship and spawning. Most of the behaviors observed in Guadalupe Bass are expressed in Smallmouth bass. Behavioral isolation is non-existent between the two species. Compounding the lack of behavioral isolation mechanisms Guadalupe Bass display a similar color pattern during courtship and spawning which is similar to observations made in Smallmouth Bass during courtship and spawning. Males of both species become dark in coloration, and their vertical barring fades, while females become lighter in coloration overall and their vertical barring intensifies to black in coloration. Color pattern recognition is known to affect mate choice in other species of fish (Williams and Mendelson 2011). Especially in species that share a common ancestry.

Total body length is a reliable predictor of female mate choice in Smallmouth Bass (Ridgway et al. 1987; Hanson and Cooke 2009). If total length be a reliable predictor of female mate choice in Guadalupe Bass then Smallmouth Bass males would be at a significant advantage over male Guadalupe Bass in systems where they occur together. Guadalupe Bass attain a smaller mean size than Smallmouth Bass (Warren 2009). Should female mate choice in Guadalupe Bass rely on total length then Smallmouth Bass males hold a size advantage over male Guadalupe Bass. Female Guadalupe Bass may be selecting Smallmouth Bass at greater frequencies than their own species and thus perpetuating the persistence of hybrids. Large male Smallmouth Bass nest earlier in the spawning season (Ridgway et al. 1991) and thus gain access to nesting sites earlier than perhaps Guadalupe Bass males begin to seek out nesting sites.

There is no pre-determined sequence in Guadalupe Bass that elicits egg deposition. The courtship and spawning sequence of Guadalupe Bass displays plasticity and there is no hard wired behavior to elicit egg deposition in the female. Male Smallmouth Bass have the same behavioral tendencies as male Guadalupe Bass and therefore are able to successfully mate with female Guadalupe Bass. Behavioral isolation is non-existent between these two at one time allopatric species.

Guadalupe Bass males were observed to monopolize nesting mats and females in the hatchery environment. This differs from observations of Largemouth Bass courting in the hatchery environment, where all available nesting mats are guarded by male Largemouth Bass. There is a high monopolization potential in Guadalupe Bass in the hatchery environment. The effective population size in the hatchery will be reduced if a

relatively few number of males are securing access to the majority of females used as brood stock for restoration efforts. Reduced variation in the offspring produced for stocking reduces the potential for successful survival and future recruitment and persistence of pure strain Guadalupe Bass.

This monopolization potential appears to exist in the natural environment (Gorman Creek). As discussed above nesting site habitat used by Guadalupe Bass are similar to that used by Smallmouth Bass. Large Smallmouth Bass that acquire nesting sites and hold nesting sites that would otherwise be used by male Guadalupe Bass are likely to acquire multiple matings with females of either species that approach the nesting site. If total length and/or color pattern be reliable predictors of mate choice in female Guadalupe Bass there exists the significant effect that Smallmouth Bass males secure exclusive access to females of either species and monopolize nesting sites.

5.2 Future Directions

Males must defend a resource (nesting site) we would expect females to aggregate around these resources (males and nesting sites). This would increase the variance in mating success in males and increase the opportunity for sexual selection (Shuster and Wade 1991; Shuster and Wade 2003). In systems where Guadalupe Bass and Smallmouth Bass co-occur the occupation and retention of suitable nesting sites by male Smallmouth Bass that are large and are able to attract females of either species would unequally distribute the majority of successful matings towards male Smallmouth Bass. This would result in the continued persistence of hybrids systems where Smallmouth Bass were introduced and established.

The observation that Guadalupe Bass males and females adopt differing color patterns raises the question as to what the purpose of this differentiation in coloration between sexes. This color pattern change is temporary and occurs during courting and spawning activity. Is mate choice selection based on the coloration displayed by individuals in a system? Smallmouth Bass males and females display a similar coloration pattern during courtship (Ridgway et al. 1989). Secondly, is this a potential miscue responsible for the high degree of hybridization that has been observed in systems where Smallmouth Bass were introduced? Applied studies of female mate choice between a conspecific, hybrid, and a heterospecific male could be conducted to determine if color is a predictor of female mate choice. It has also been determine in Smallmouth Bass that total length and body stoutness are reliable predictors of female mate choice. These physical characteristics could either be working independent of each other or interdependently to influence female mate choice. Species isolating mechanisms are non-existent between Guadalupe Bass and Smallmouth Bass. Before the introduction of Smallmouth Bass into Texas the ranges never overlapped, and thus an isolating mechanism was not evolved. Future studies need to address whether color pattern and/or body size are reliable predictors of female mate choice in Guadalupe Bass.

The observations of multiple individuals attempting to court within a single nest mat/site was an unexpected behavior. It appeared as though these multiple individual events consisted of a single male and two or more females. The function of this behavior is uncertain at this time. A possible hypothesis is that females are copying each other's behavior with respect to selecting a male. Female copying, defined as a female's

tendency to copy the behavior of other females (Shuster and Wade 1991) is a method of information sharing and works well in systems where resources (nesting sites) are distributed in a patchy manner throughout the environment (Shuster and Wade 2003). Nesting sites may be distributed patchily throughout river environments and female copying would benefit females by shortening their time spent searching for a suitable male Guadalupe Bass that is defending a suitable nesting site. If female mate choice are dependent on color pattern and/or total length then female Guadalupe Bass are more likely to select large male Smallmouth Bass. Females are choosing incorrectly with regards to their species and compounding this effect is the reliance of other females mate choice by females that are using the female copying strategy for mate selection.

The monopolization of nesting mats and females in the hatchery setting by male Guadalupe Bass is of concern for maintaining the effective population size of hatchery raised fish for restoration efforts. Several recommendations to ensure a large effective population size would be to: divide the raceways into smaller sections and maintain a 1:2 male to female ratio, employ a rotation system to ensure that all males and females are mated to the each other, and using "blinding" structures to break visual contact between males holding nesting mats. These recommendations are intended to maximize and maintain the effective population size of pure strain hatchery produced Guadalupe Bass.

Future studies of the Guadalupe Bass should investigate the following concepts to better understand the mechanisms behind hybridization with Smallmouth Bass: the importance of coloration and body size of the male as reliable predictors of female mate choice, the extent of monopolization of nesting sites in the hatchery and natural

environments, and the function of multiple individuals courting simultaneously as observed in the hatchery and natural environments.

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APPENDIX A

FIGURES

Figure 1. Diagram of A.E. Wood Hatchery Raceway # 2 Set-Up

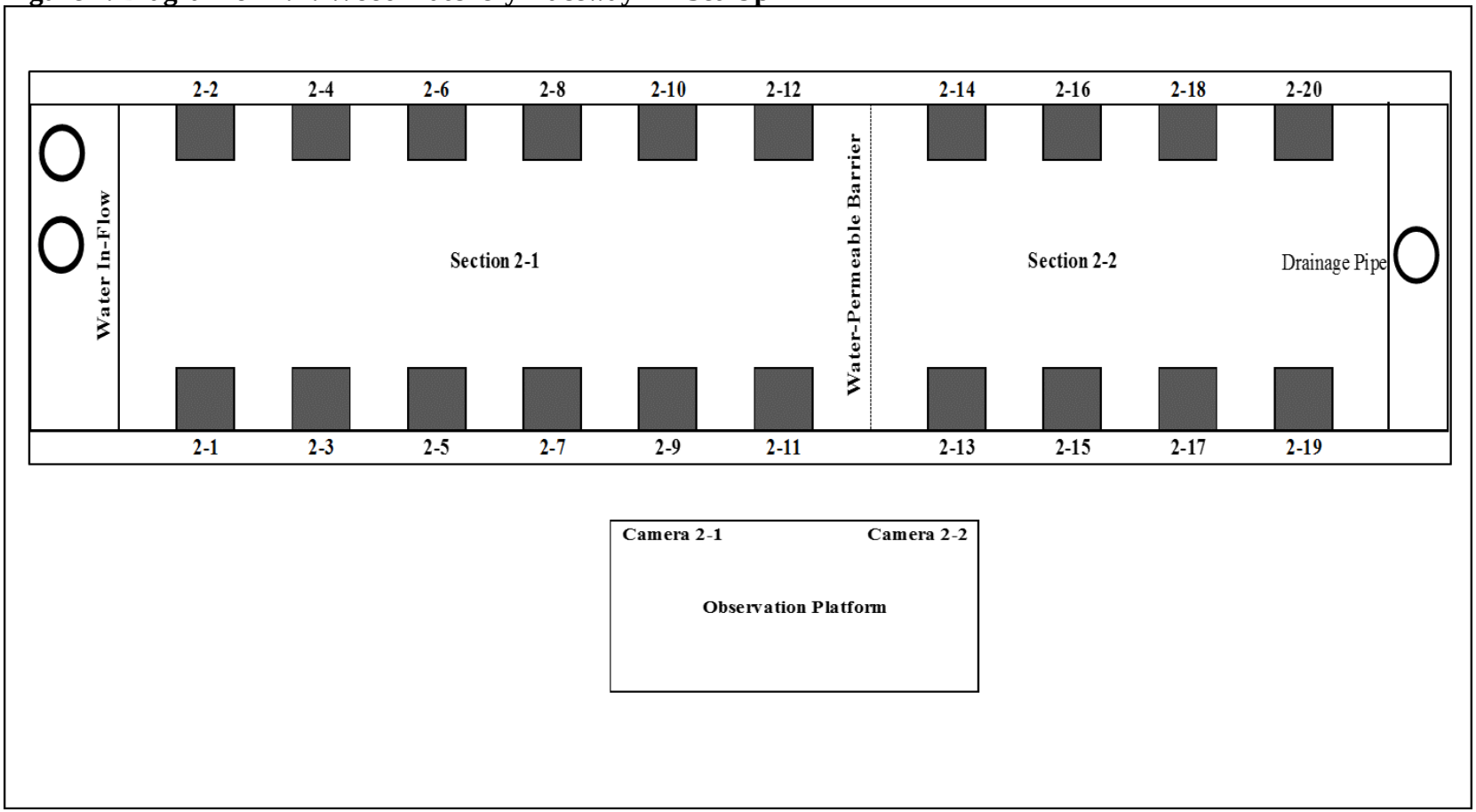


Figure 2. Nesting Sites Observed in Gorman Creek: Spring 2013.

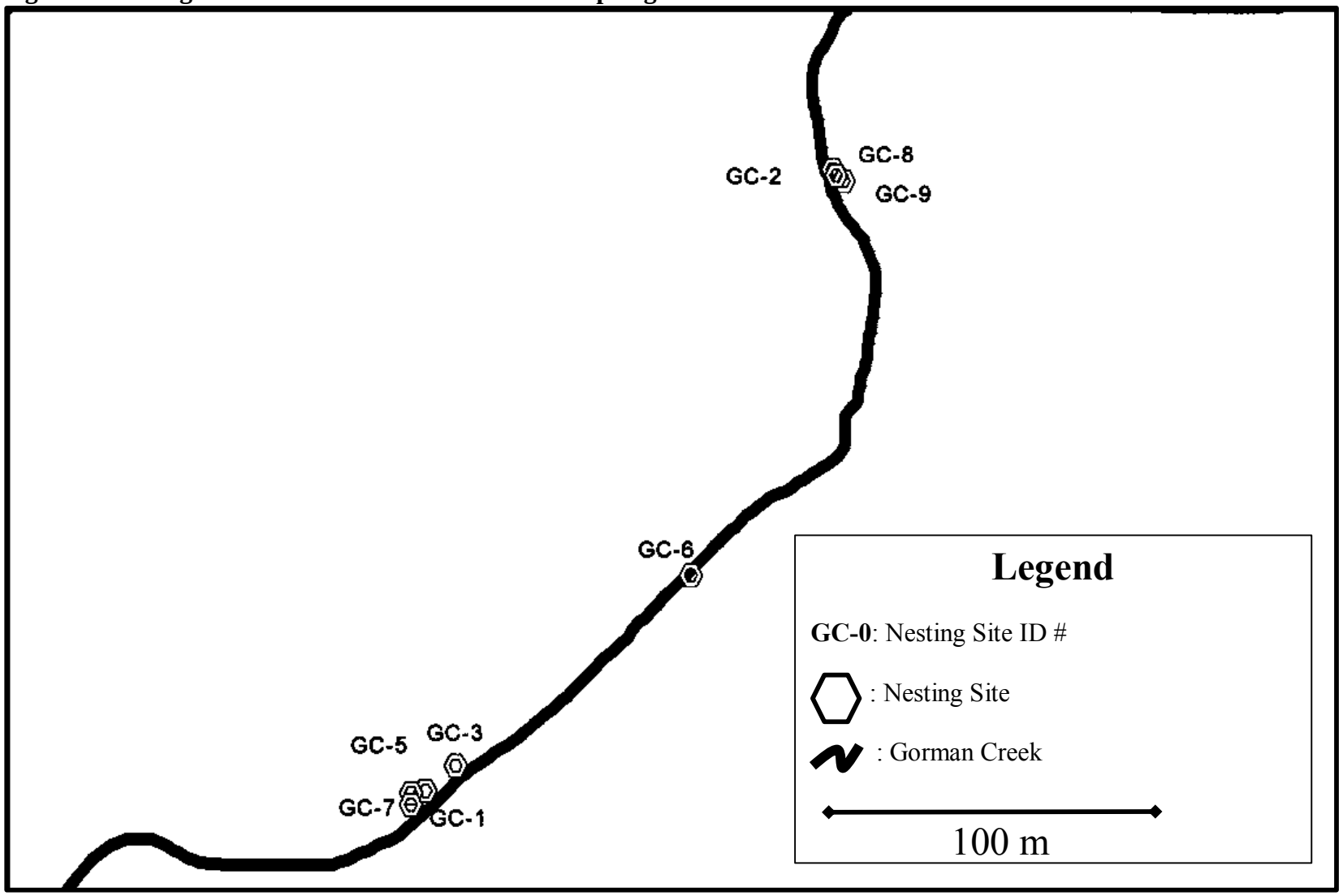


Figure 3. Length Frequency Distribution of Adult Guadalupe Bass Individuals in Gorman Creek and Pedernales River.

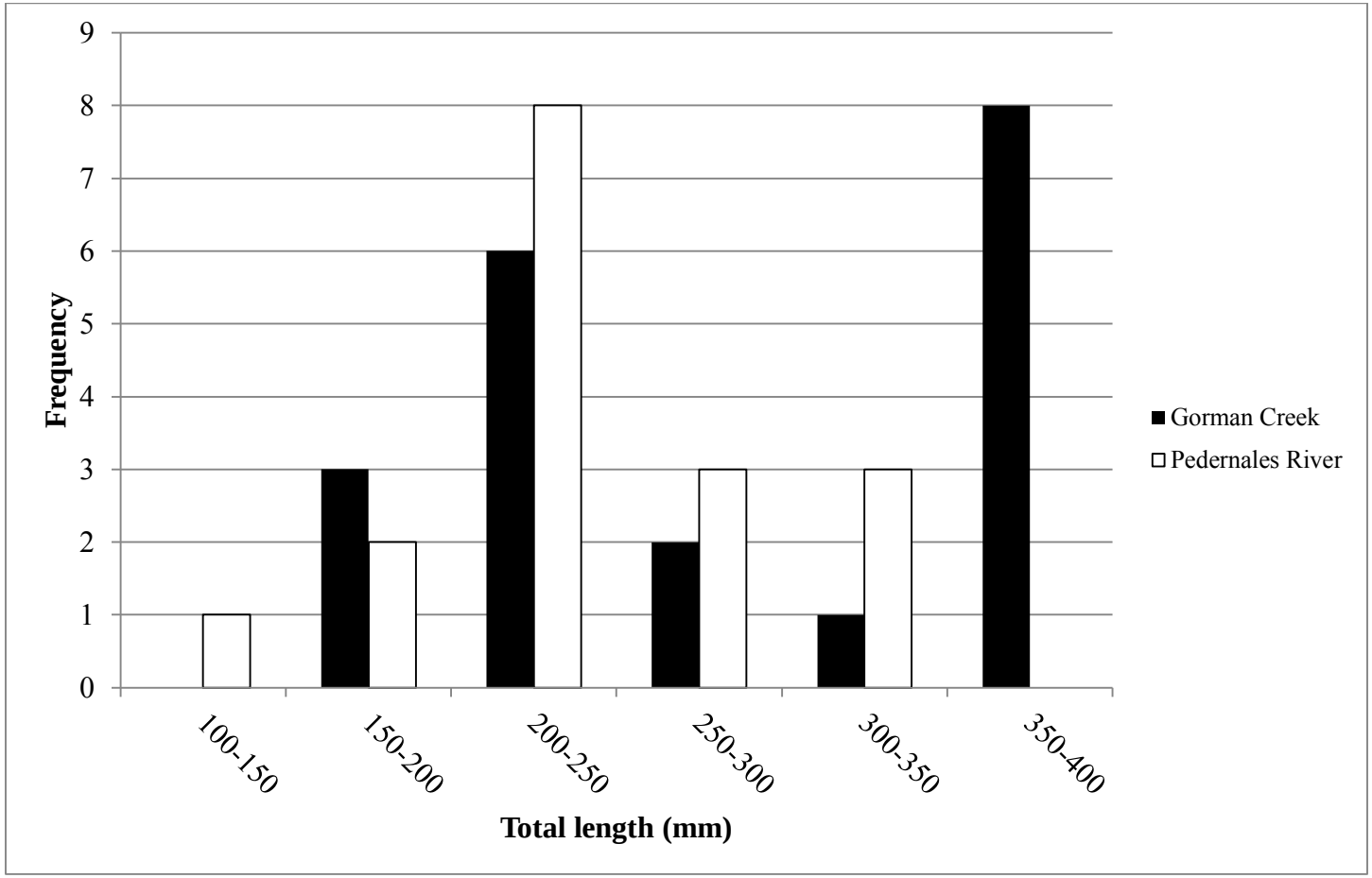


Figure 4. Frequency counts of the number of observed Guadalupe Bass occupying nesting mats during scan video recordings at A.E. Wood Hatchery.

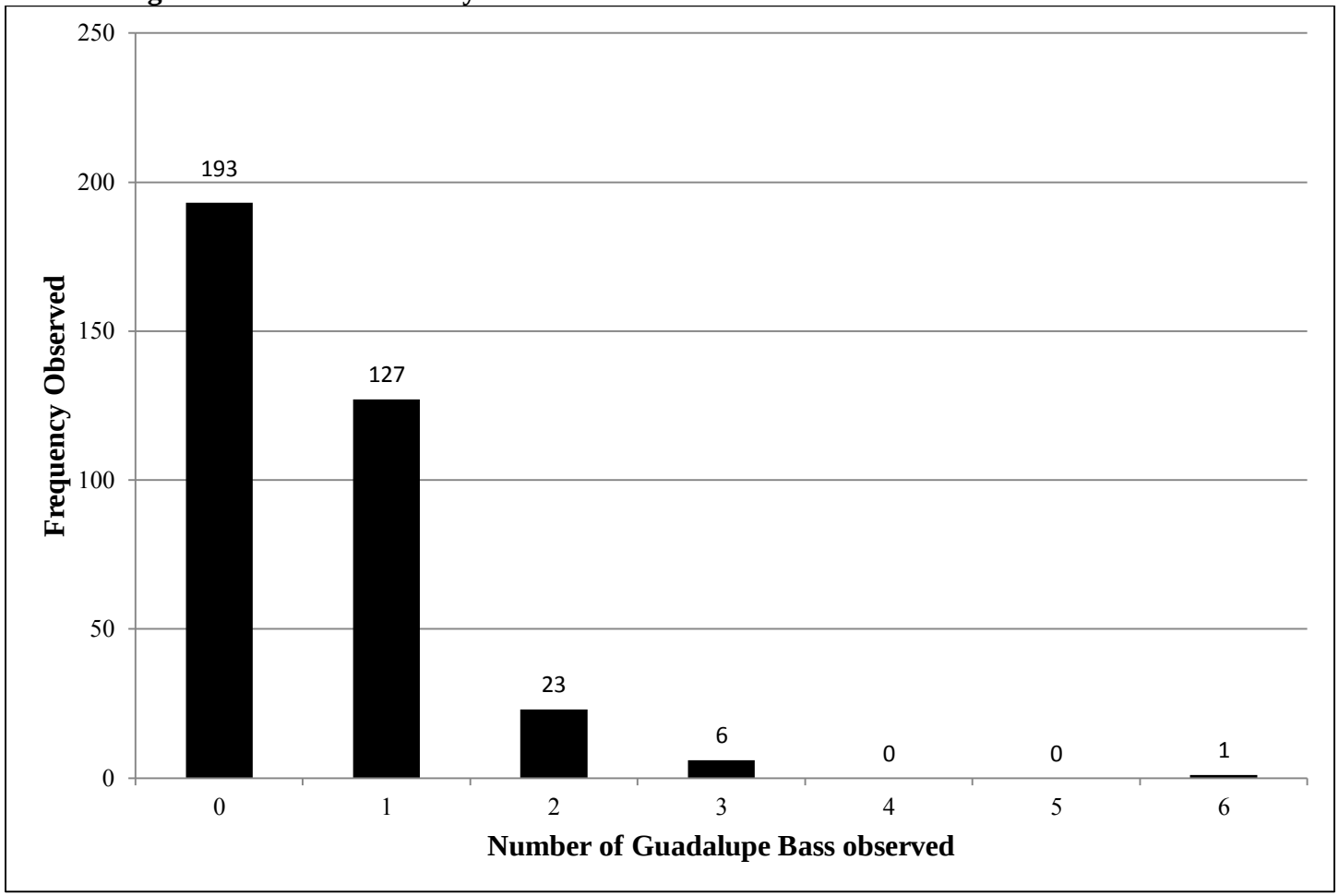


Figure 5. Proportional Distribution of Observed Activity States in Guadalupe Bass at Gorman Creek. Significant differences in the observed frequency of activity states observed are marked with an asterisk (z-score > 1.96 at 0.05 alpha level).

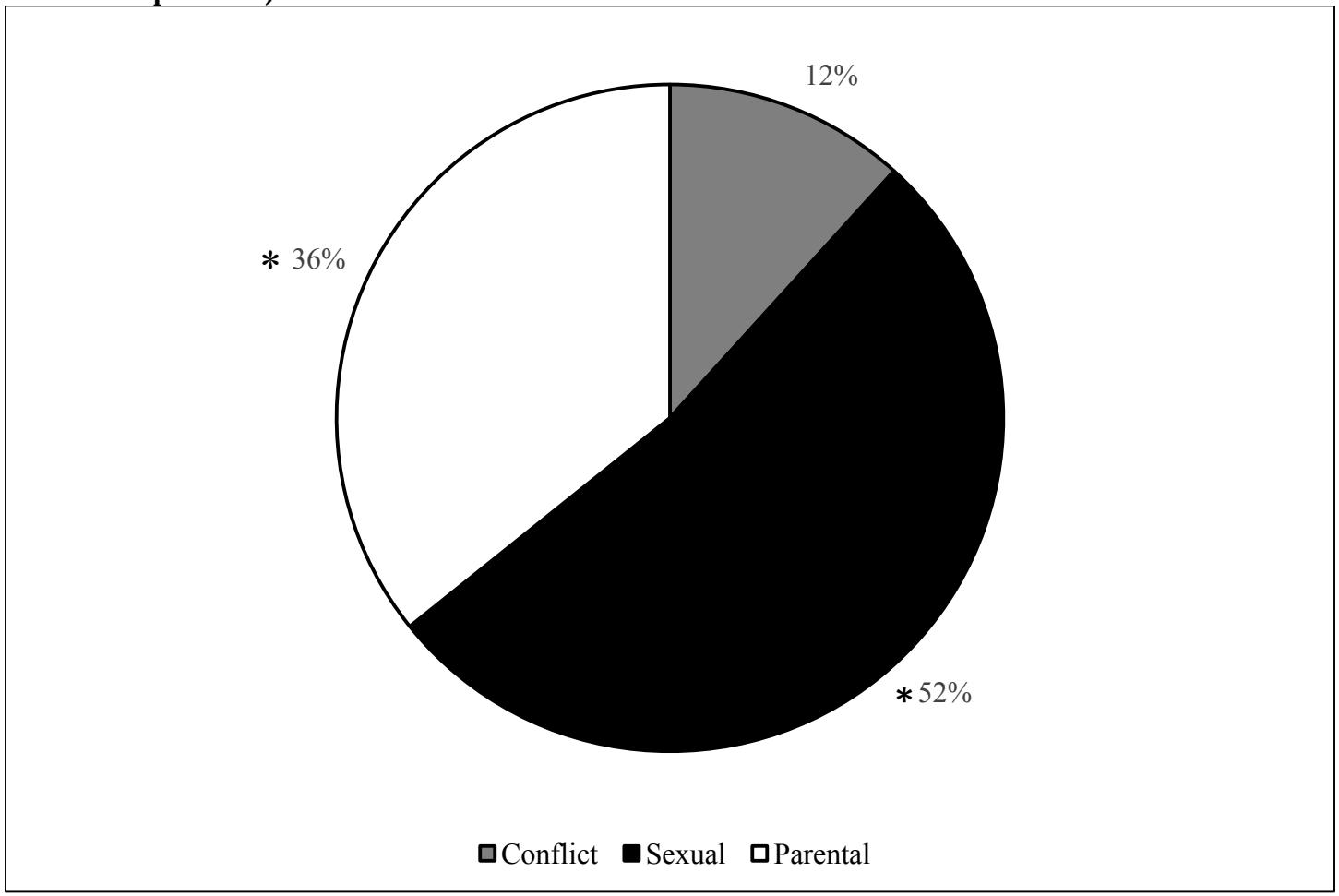


Figure 6. Proportional Distribution of Observed Activity States at A.E. Wood Hatchery. Significant differences in the observed frequency of activity states observed are marked with an asterisk (z-score > 1.96 at 0.05 alpha level).

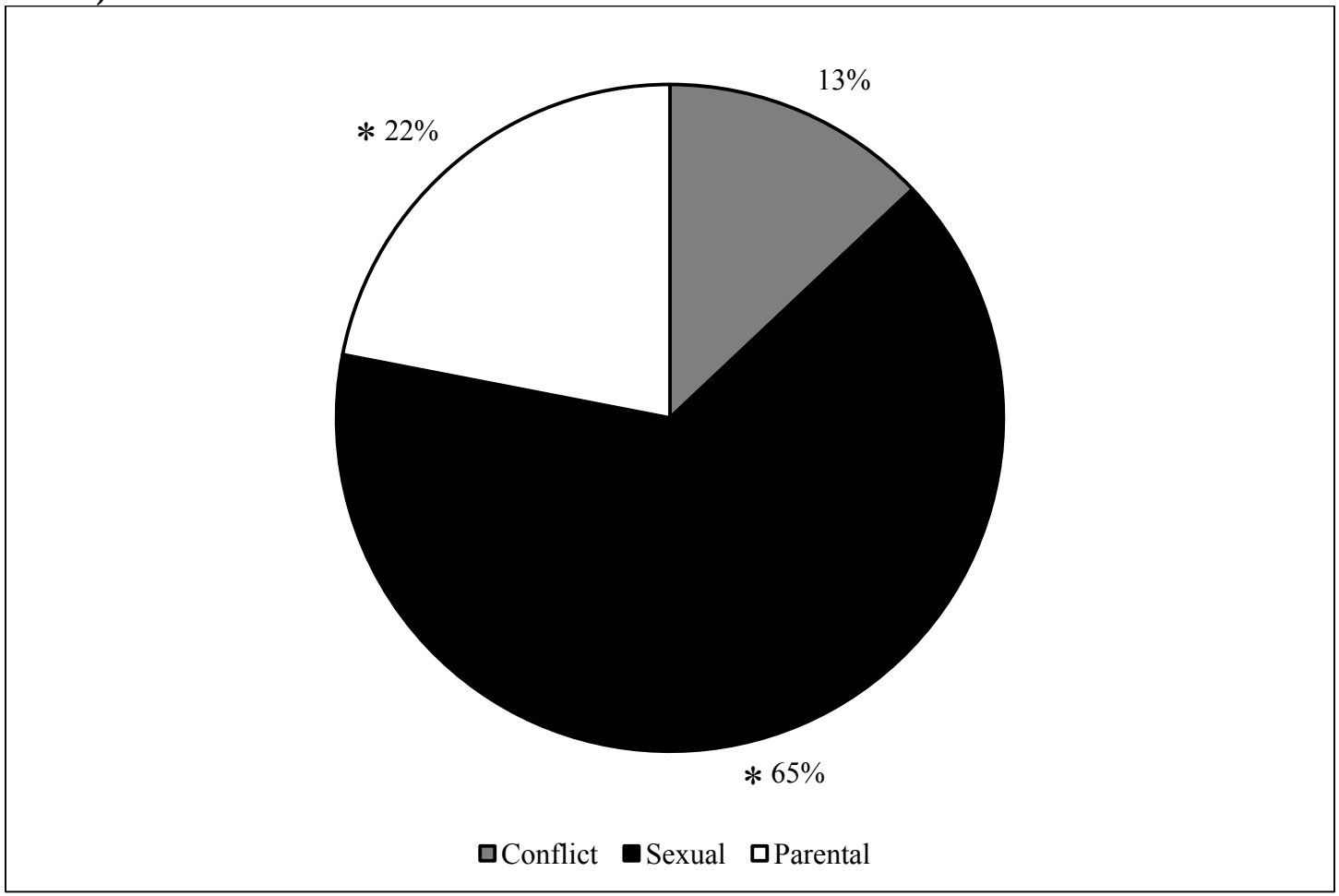


Figure 7. Proportional Distribution of Observed Activity States in Guadalupe Bass (2-2). Significant differences in the observed frequency of activity states observed are marked with an asterisk (z-score > 1.96 at 0.05 alpha level).

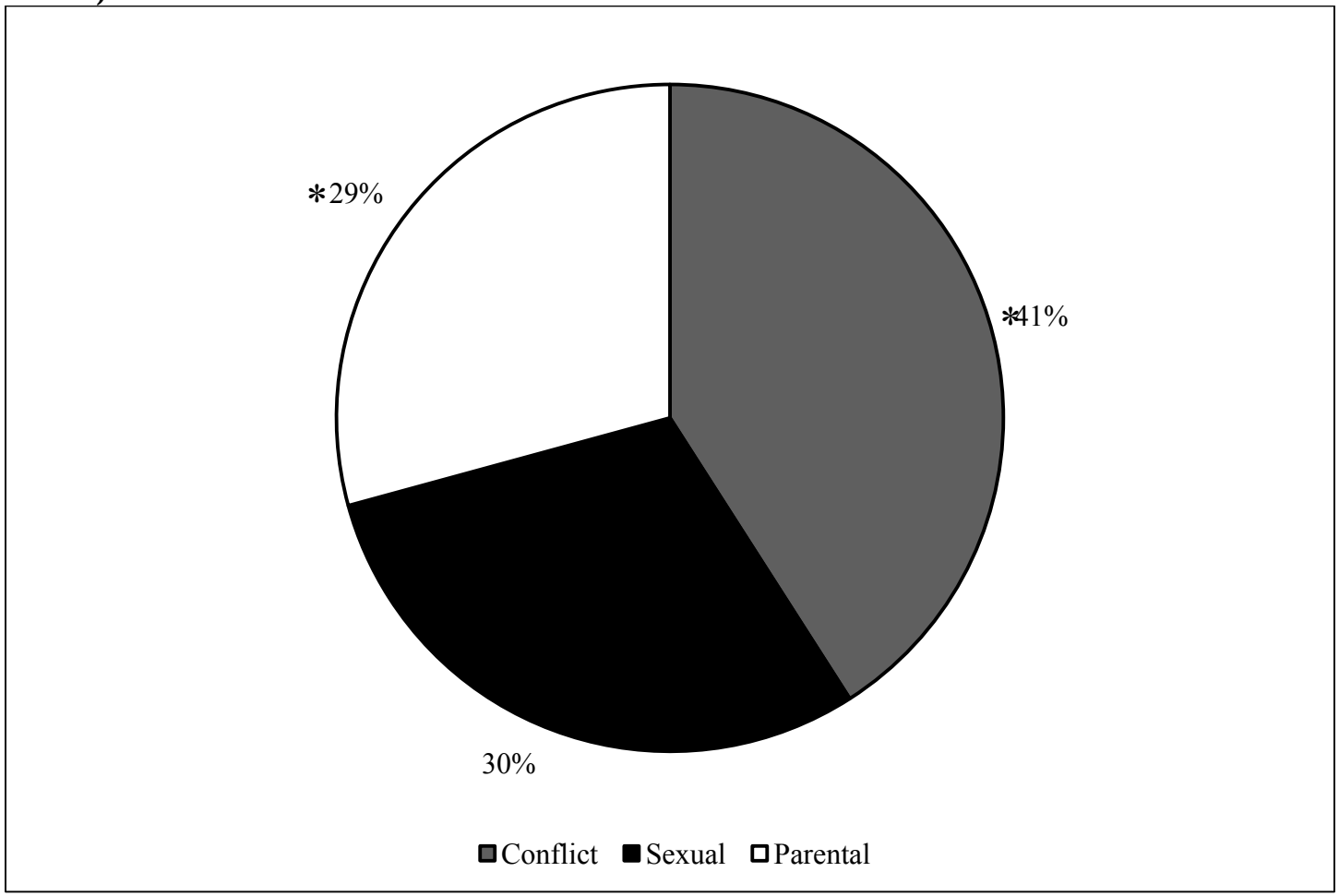


Figure 8. Proportional Distribution of Observed Activity States in Guadalupe Bass (2-10). Significant differences in the observed frequency of activity states observed are marked with an asterisk (z-score > 1.96 at 0.05 alpha level).

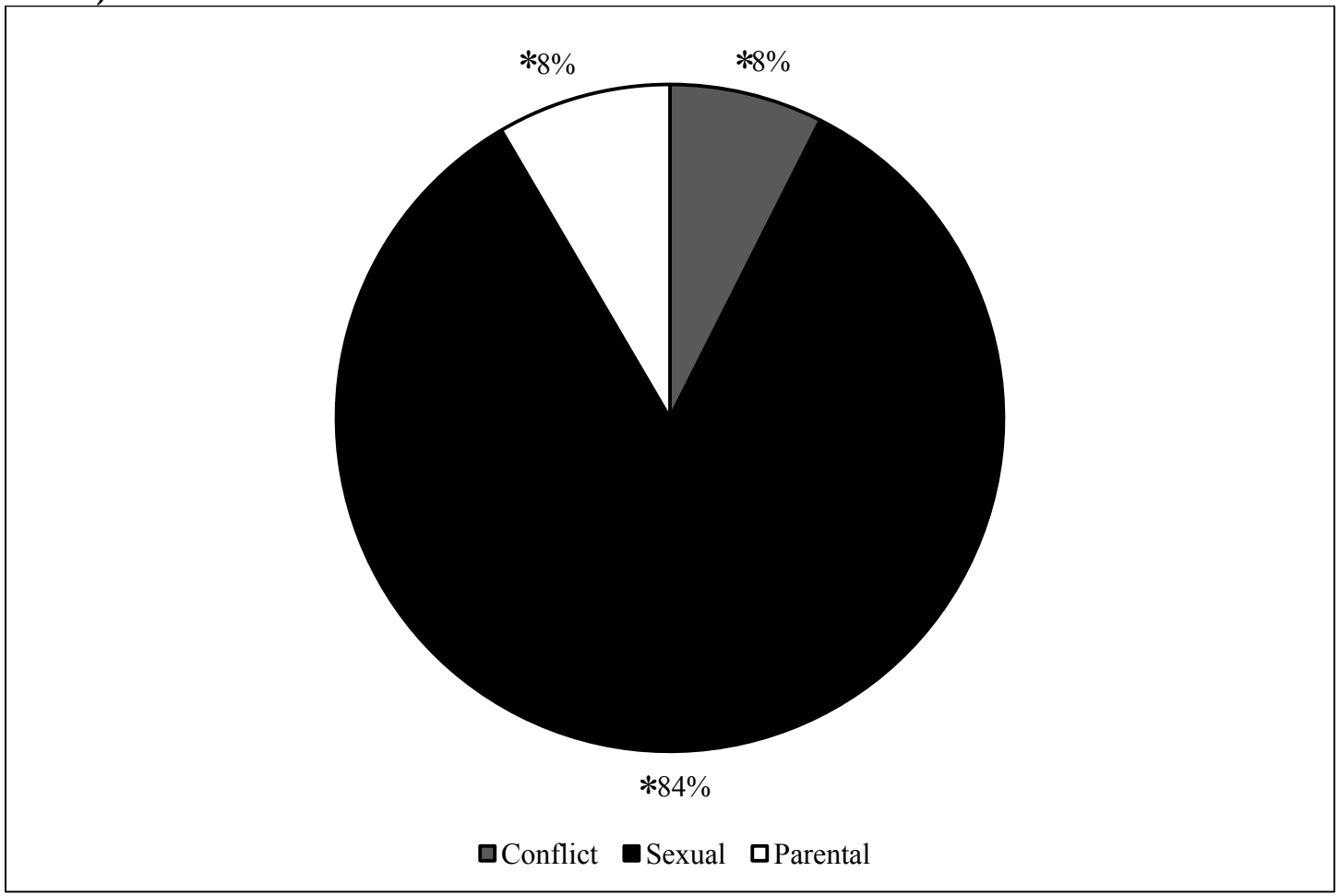


Figure 9. Proportional Distribution of Observed Activity States in Guadalupe Bass (2-14). Significant differences in the observed frequency of activity states observed are marked with an asterisk (z-score > 1.96 at 0.05 alpha level).

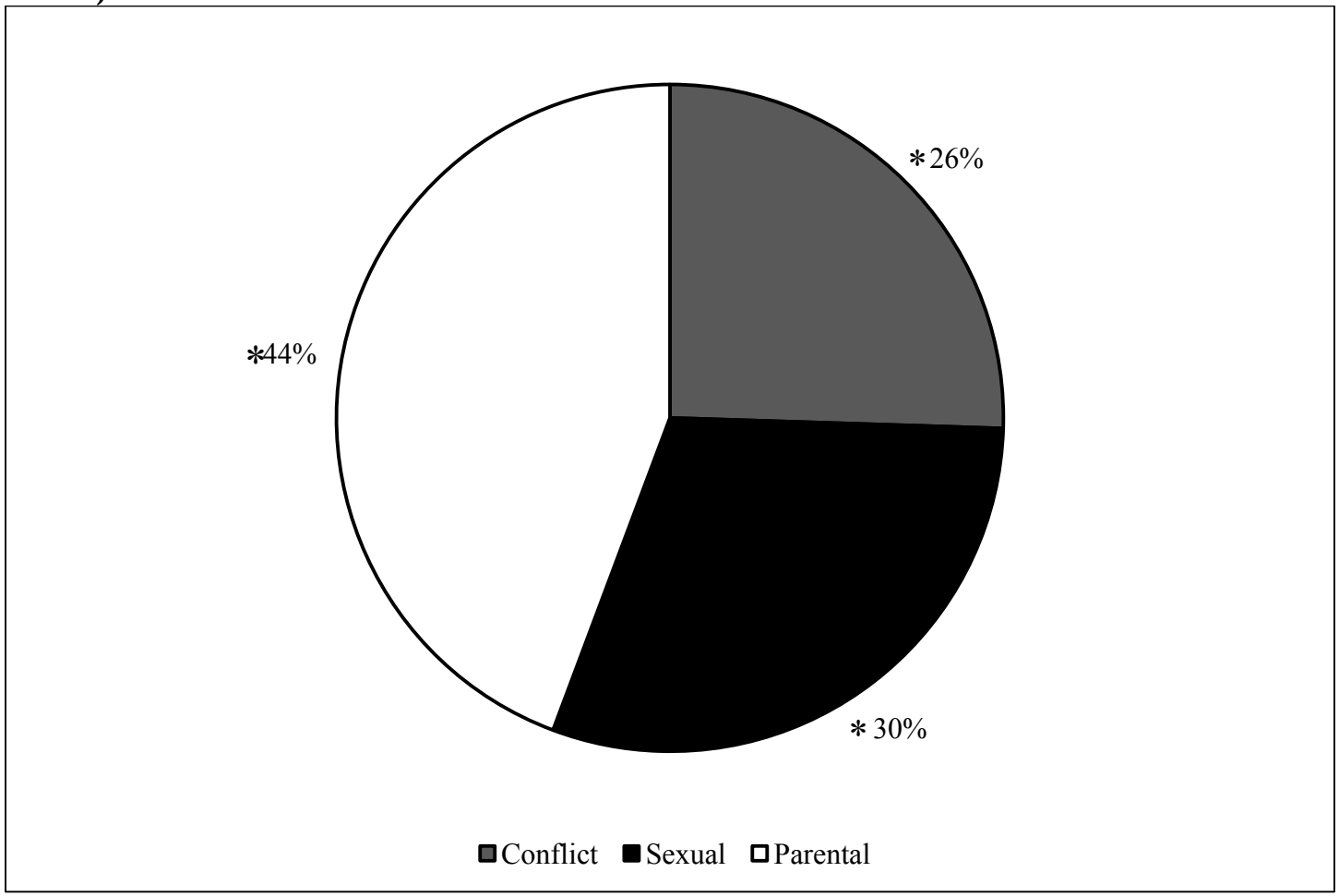


Figure 10. Proportional Distribution of Observed Activity States in Guadalupe Bass (2-18). Significant differences in the observed frequency of activity states observed are marked with an asterisk (z-score > 1.96 at 0.05 alpha level).

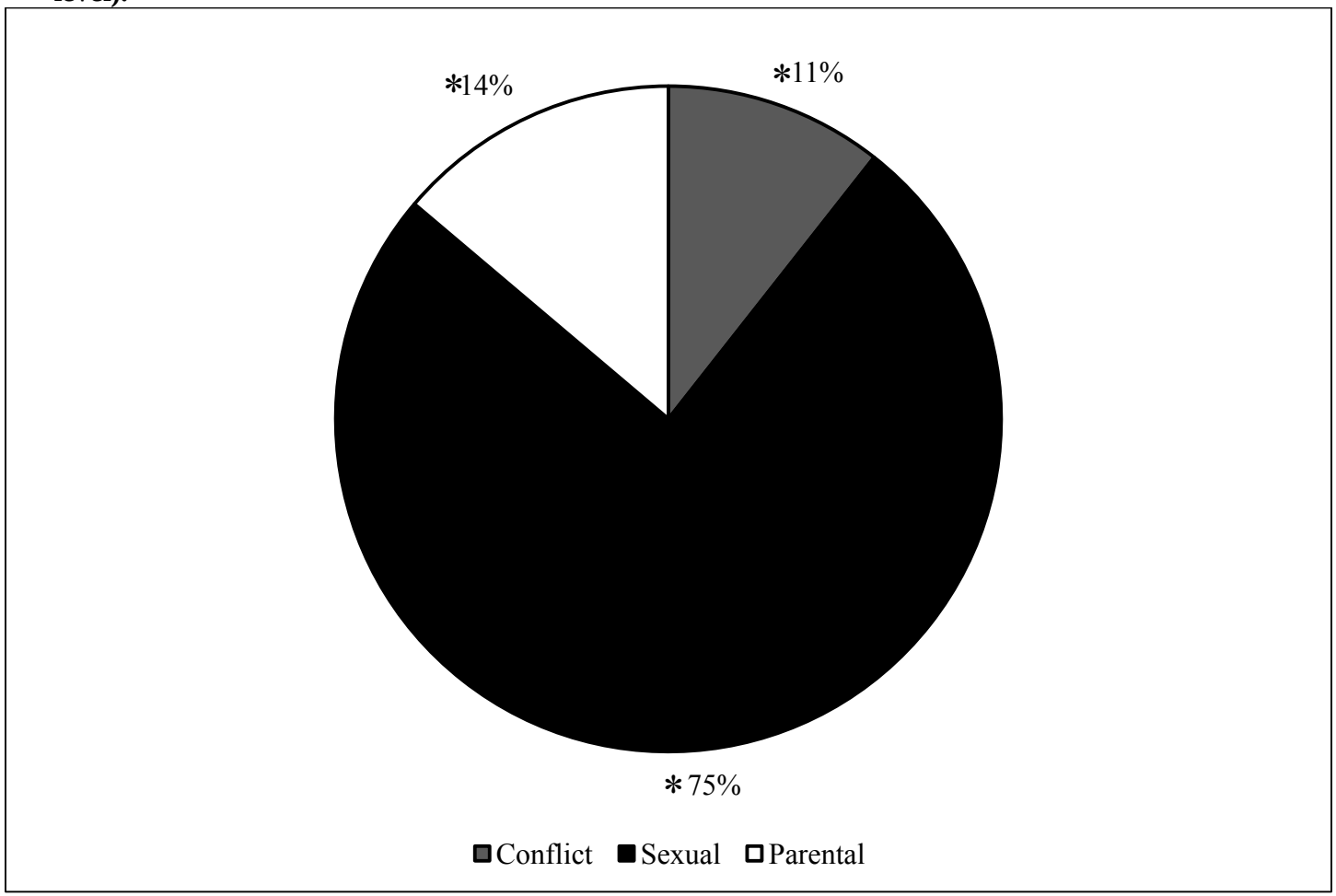


Figure 11. Proportional distribution of courting, spawning and parental care behaviors of male Guadalupe Bass at Gorman Creek. Significant differences in the observed frequency of male behaviors observed are marked with an asterisk (z-score > 1.96 at 0.05 alpha level).

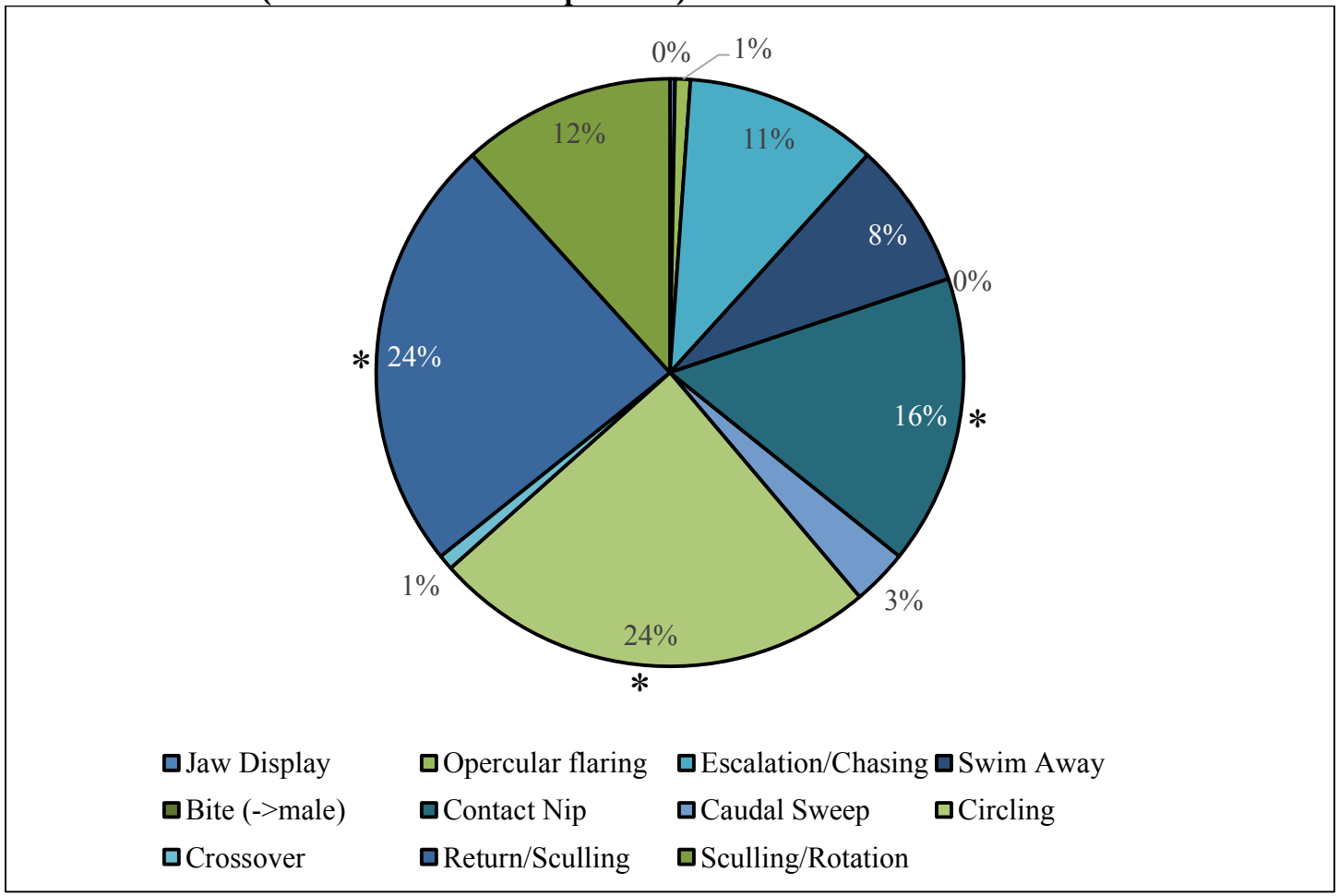


Figure 12. Proportional distribution of courting and spawning behaviors of female Guadalupe Bass at Gorman Creek Significant differences in the observed frequency of female behaviors observed are marked with an asterisk (z-score > 1.96 at 0.05 alpha level).

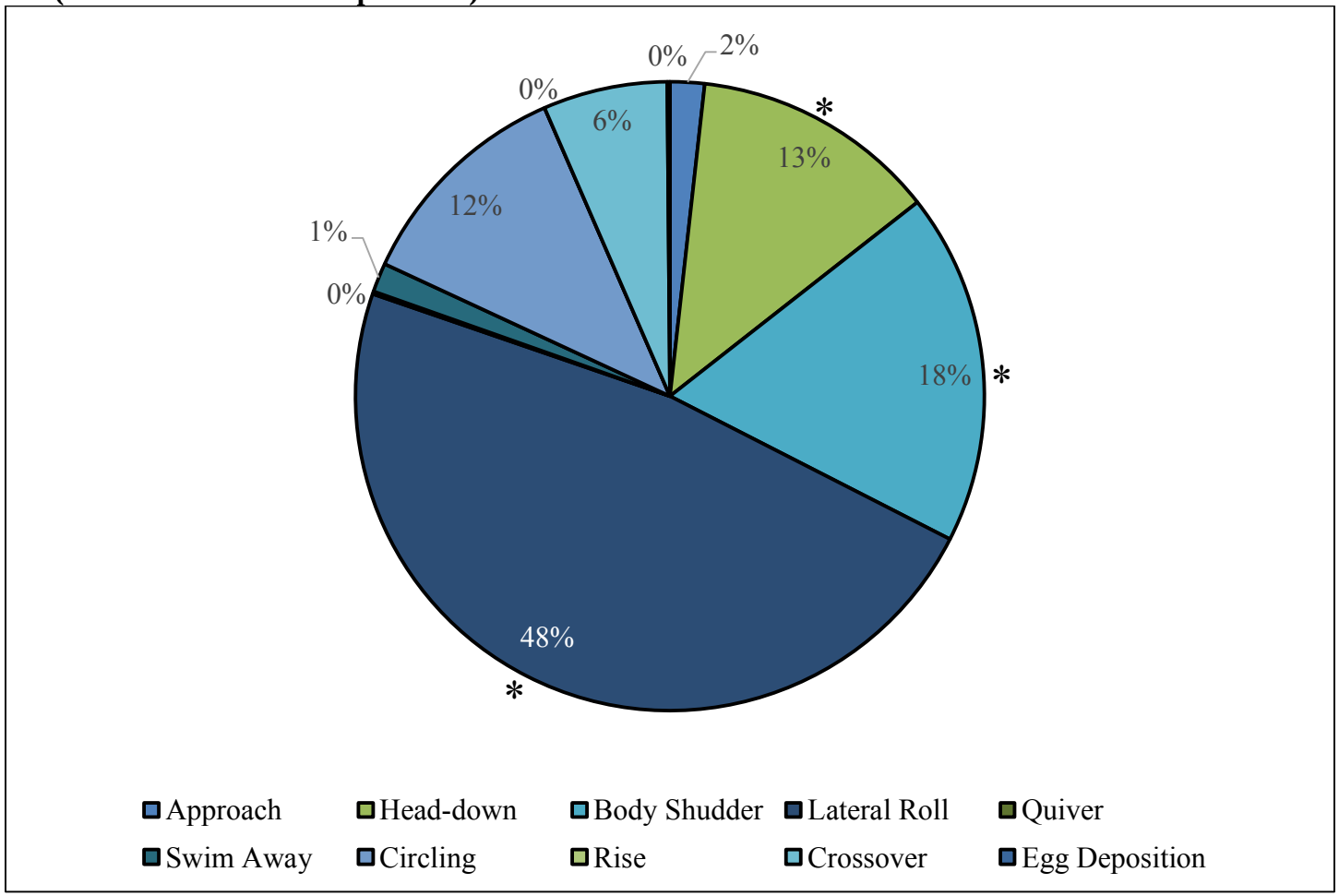


Figure 13. Proportional distribution of observed courting, spawning, and parental care behaviors of male Guadalupe Bass at A.E. Wood Hatchery. Significant differences in the observed frequency of male behaviors observed are marked with an asterisk (z-score > 1.96 at 0.05 alpha level).

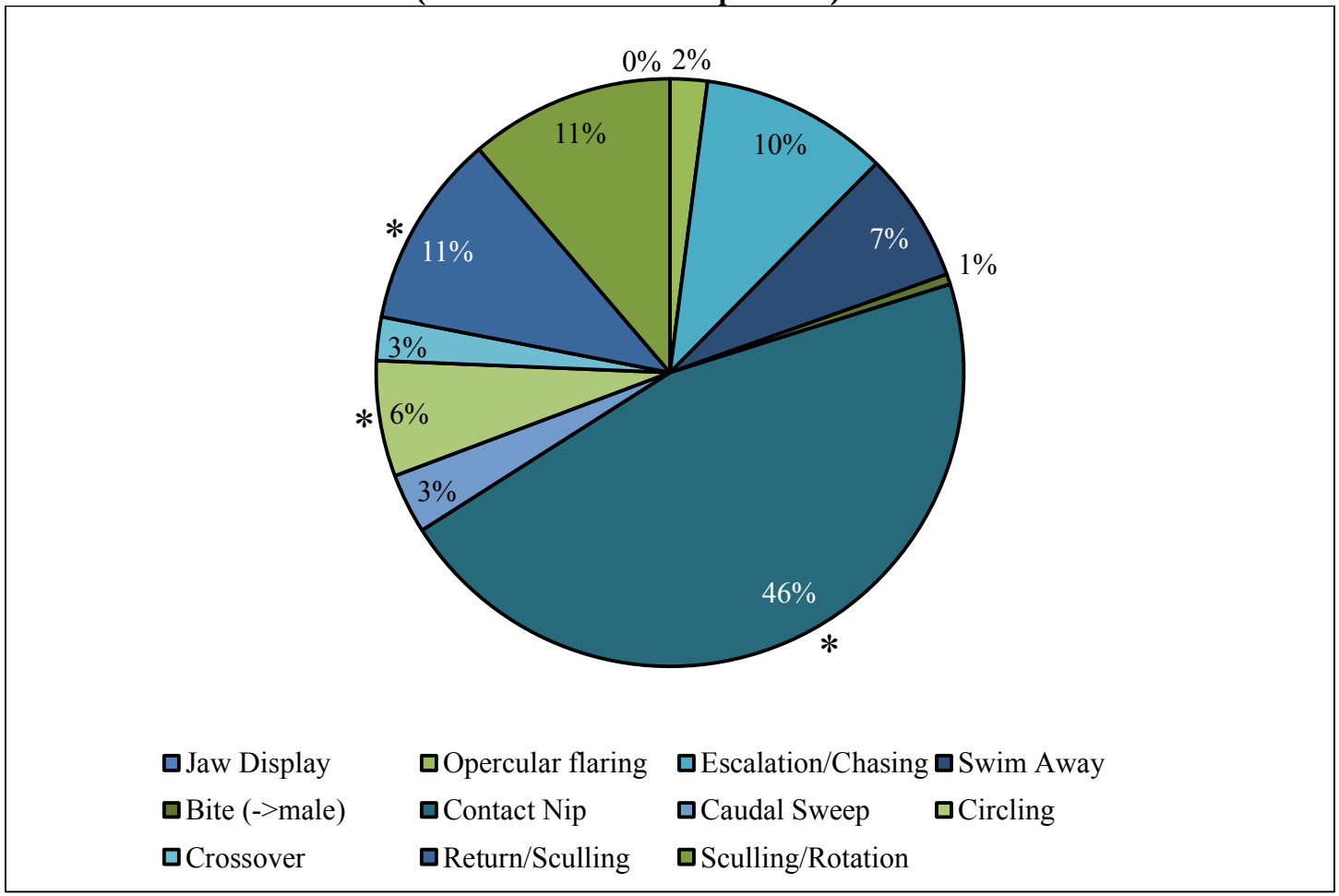


Figure 14. Observed frequencies of observed courting and spawning behaviors of female Guadalupe Bass at A.E. Wood Hatchery. Significant differences in the observed frequency of female behaviors observed are marked with an asterisk (z-score > 1.96 at 0.05 alpha level).

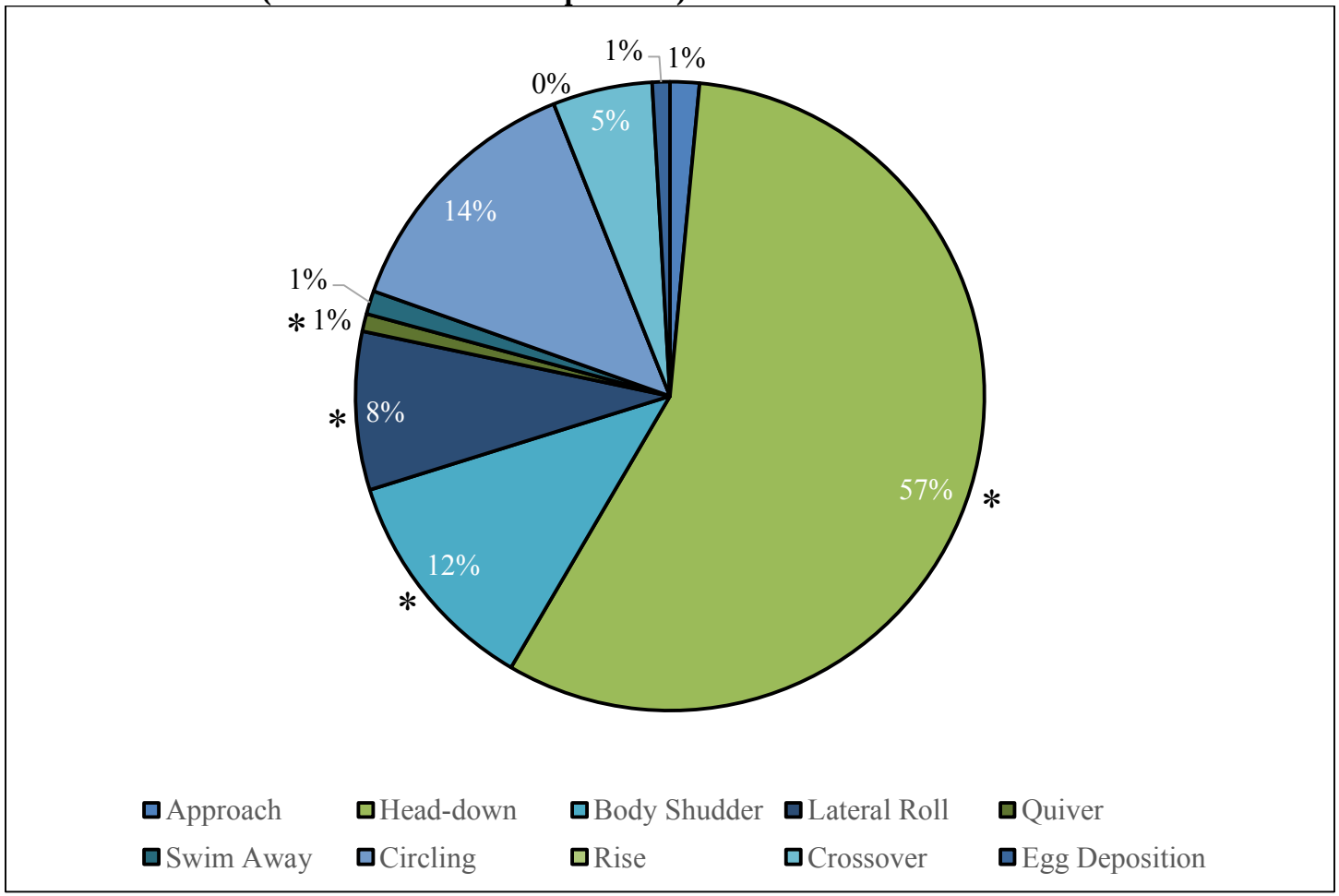


Figure 15. Kinematic diagram of male to male behaviors at Gorman Creek

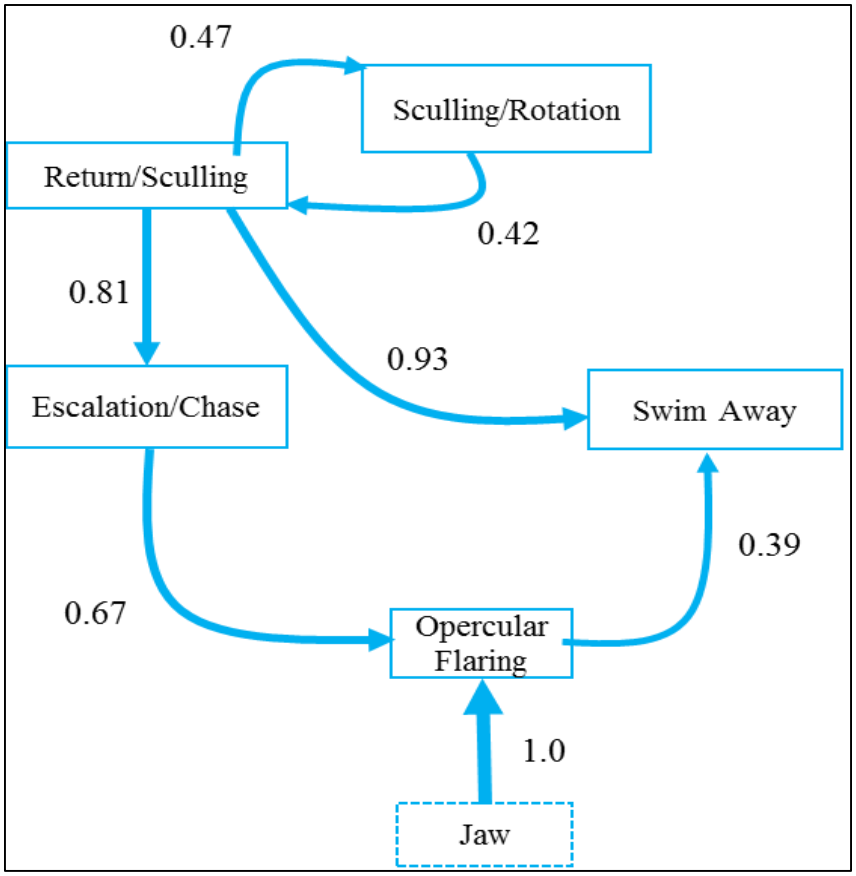


Figure 16. Kinematic diagram of male to male behaviors at A.E. Wood Hatchery

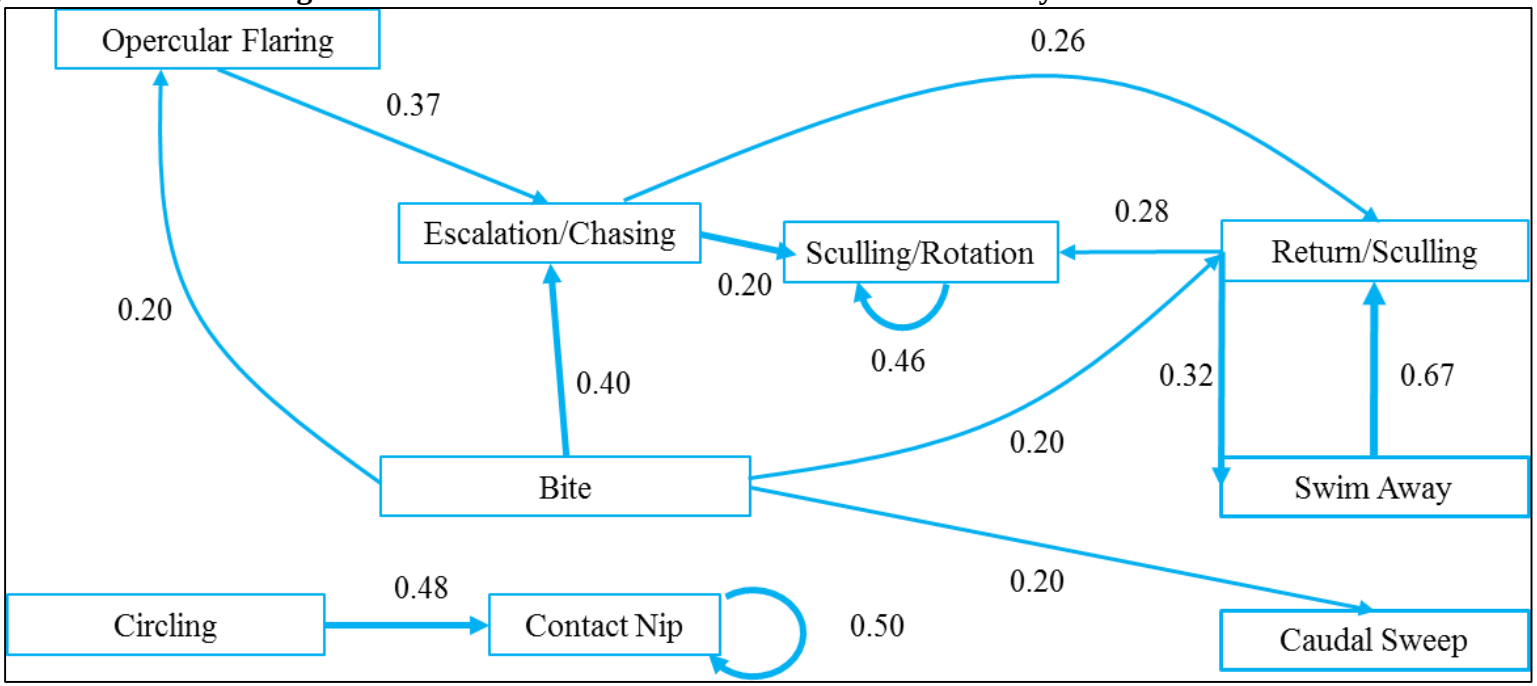


Figure 17. Kinematic diagram of female to female behaviors at Gorman Creek

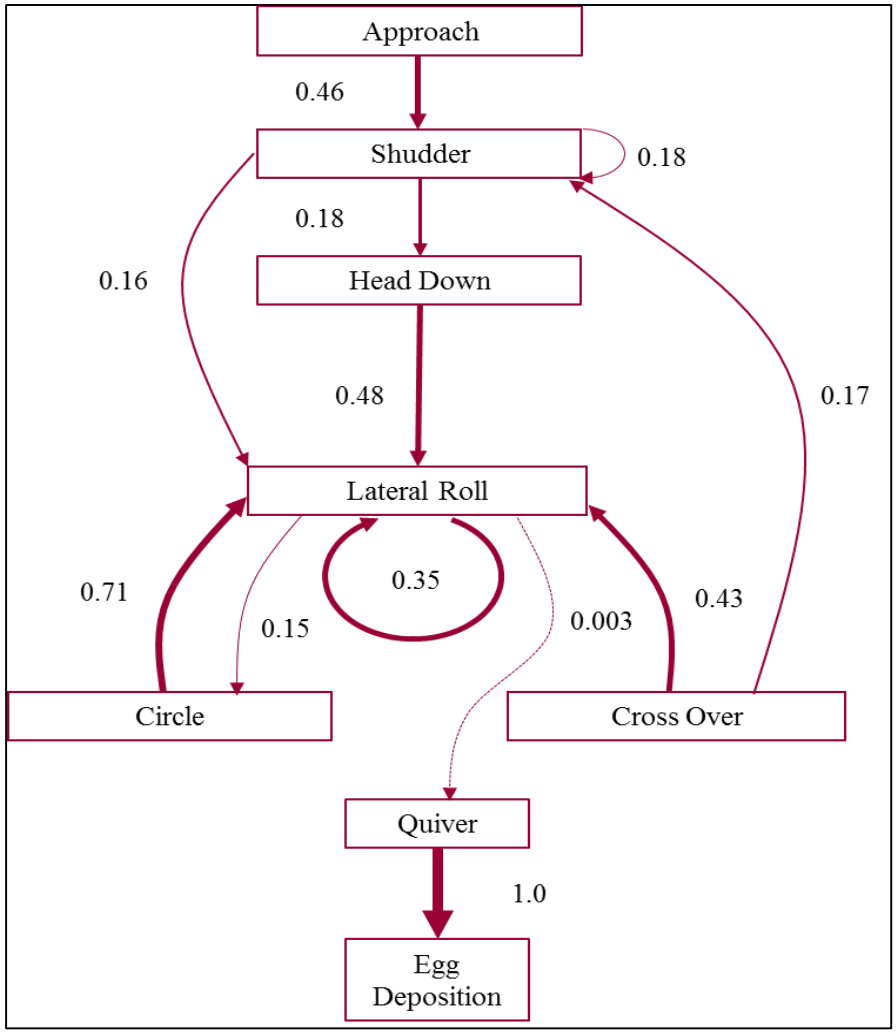


Figure 18. Kinematic diagram of female to female behaviors at A.E. Wood Hatchery

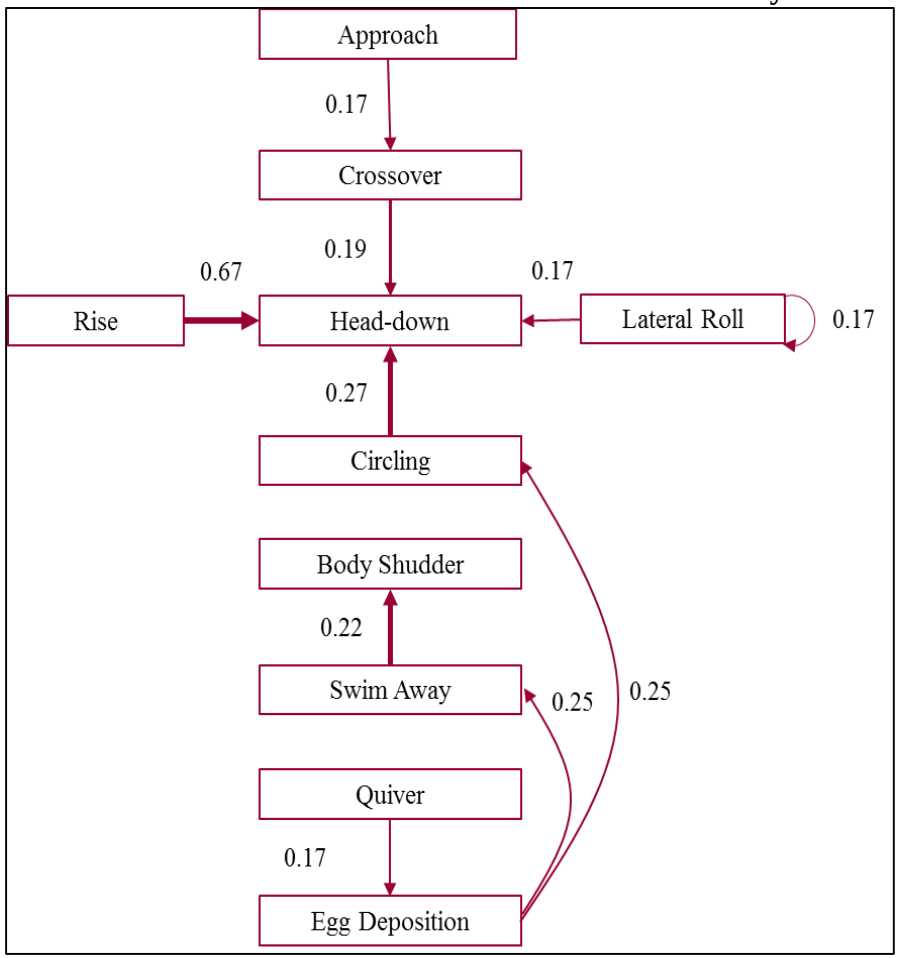


Figure 19. Prototypical courtship and spawning sequence of Guadalupe Bass mating pair in Gorman Creek.

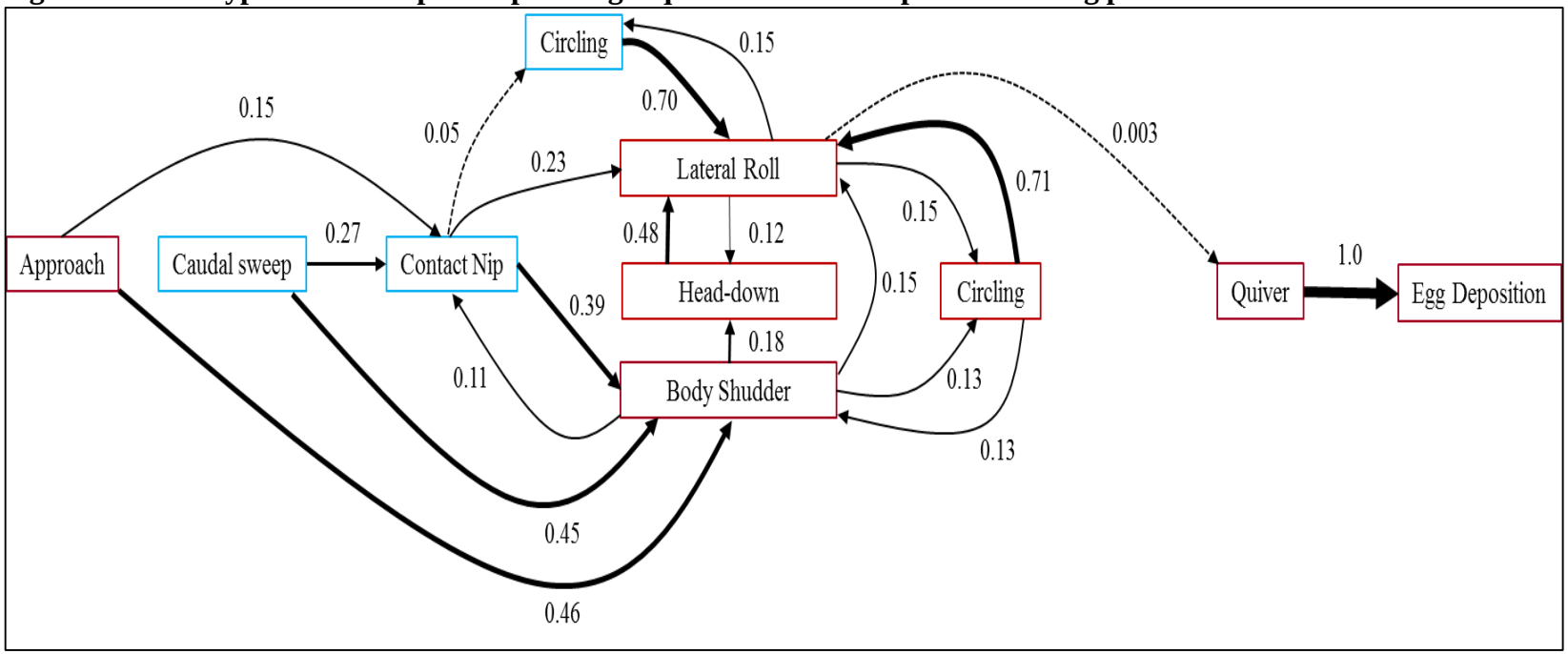
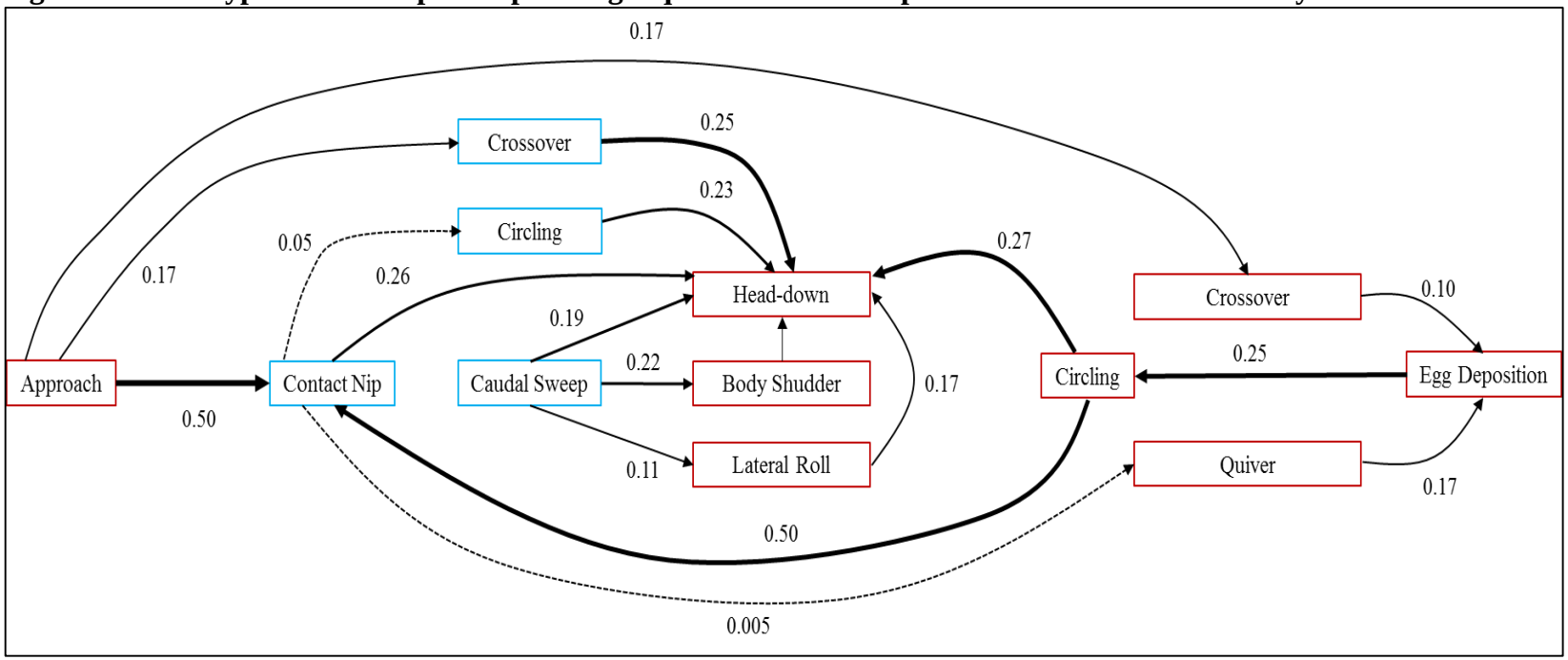


Figure 20. Prototypical courtship and spawning sequence of Guadalupe Bass at A.E. Wood Hatchery



APPENDIX B

TABLES

Table 1. Ethogram of Guadalupe Bass courting, spawning, and parental care.

Activity State	Description of subcategories	Indicator behaviors
Conflict	Assess: Males position themselves head to head or alongside each other	Jaw display, Opercular flaring
	De-escalate: Individual moves away from another fish that reduces the probability of conflict	Swim Away, Turn Away
	Escalate: Individual moves quickly towards another individual	Chase, Contact nip, Bite, Jaw locking/shoving
Sexual	Reject: Female approaches male/nest site but leaves soon after approaching. Male ignores female or chases female off nest	Approach, Swim away
	Proceptive: Female approaches and male courts female, no egg/sperm deposition	Female approaches, head-down, shudder, lateral roll swims away/chased away, rise, circling, crossover, contact nip, caudal sweep
	Receptive: Male & female complete full courtship/spawning sequence resulting in eggs deposited & fertilized in nest	Head-down, Body shudder, lateral roll, quiver & egg deposition/sperm release
Parental Care	Fanning: Male fans eggs with pectoral fins (sculling action)	Sculling action with pectoral fins directly over fertilized eggs
	Fanning with Rotation: Male fanning eggs with pectoral fins while rotating within the nest.	Sculling action with pectoral fins directly over fertilized eggs, male rotates through entire 360° field of view from nest center
	Escalation/Attack Intruders: Male moves towards chases intruders coming close to nest boundary using threat displays	Chasing/charging, gill flaring, biting

Table 2. Nesting habitat and environmental data table of nesting sites: Gorman Creek.

Nest ID	Date Found	Macrohabitat	Substrate	Depth (m)	Nest Diameter (cm)	Nearest Structure (m)	Avg. Temp C	Velocity (m/s)
GC-1	3/21/13	Pool	Gravel	0.7	36	3	20.8	-0.01
GC-2	3/21/13	Pool	Silt/Clay	0.55	37	0.4	18.2	-0.01
GC-3	3/22/13	Pool	Gravel	0.52	45	4	19.5	0
GC-5	3/23/13	Pool	Silt/Clay	0.91	70	2.4	19.9	-0.01
GC-6	3/21/13	Pool	Silt/Clay	0.5	40	0.6	19.8	0.0
GC-7	3/28/13	Pool	Gravel	0.58	50	1	20.3	0.01
GC-8	3/29/13	Pool	Silt/Clay	0.56	50	0.5	22.15	0.02
GC-9	4/10/13	Pool	Gravel	0.45	65	1	17.5	-0.01

Table 3. Guadalupe Bass egg and fry data: Gorman Creek

Nest Site ID #	Eggs Deposited	Date Young Observed	Length of Parental Care (Days)	Successful? (Yes/No)
GC-1	Yes	3/21/2013	1	No
GC-1	Yes	4/13/2013	1	No
GC-2	No	N/A	0	No
GC-3	No	N/A	0	No
GC-5	Yes	3/26/2013	22	Yes
GC-6	Yes	3/27/2013	21	Yes
GC-7	Yes	4/1/2013	17	Yes
GC-8	Yes	4/1/2013	1	No
GC-9	No	N/A	0	No

Table 4. Water Quality and Nesting Data: A.E. Wood Hatchery Raceway 2

Nest Mat ID#	Date Courting Behavior Observed	Date Eggs Picked Up	Temp °C (PM)	Temp °C (AM)	DO mg/L (AM)	DO mg/L (PM)
2-2	3/14/2013	3/15/2013	18.8	18.2	9.4	9.5
2-4	-	-	-	-	-	-
2-6	3/13/2013	3/14/2013	18.1	17.5	9.4	9.4
2-8	-	-	-	-	-	-
2-10	3/13/2013, 3/15/2013	3/14/2013, 3/16/2013	18.1,19.4	17.5, 18.9	9.4, 9.2	9.4, 11.1
2-12	-	-	-	-	-	-
2-14	3/13/2013	3/14/2013	18.1	17.5	9.4	9.4
2-16	-	-	-	-	-	-
2-18	3/13/2013, 3/15/2013	3/14/2013, 3/16/2013	18.1, 19.4	17.5, 18.9	9.4, 9.2	9.4, 11.1
2-20	-	-	-	-	-	-